ISSN 0355-1180

UNIVERSITY OF HELSINKI

Faculty of Agriculture and Forestry

Department of Food and Nutrition

EKT Series 1972

EFFECT OF INTERFACIAL AND MATRIX PROPERTIES OF MICROENCAPSULATED FLAXSEED OIL ON OXIDATIVE STABILITY DURING STORAGE AT DIFFERENT RELATIVE HUMIDITIES

Ma. Heidi Vicente-Gabucan

Helsinki 2020

HELSINGIN YLIOPISTO — HELSINGFORS UNIVERSITET — UNIVERSITY OF HELSINKI

Tiedekunta/Osasto — Fakultet/Sektion — Faculty	Laitos — Institution — Department	
Faculty of Agriculture and Forestry	Department of Food and Nutrition	
Tekijä — Författare — Author		
Ma. Heidi Vicente Gabucan		
Työn nimi — Arbetets titel — Title		
Effect of interfacial and matrix properties of microencapsulated flaxseed oil on oxidative stability during storage at different relative humidities		
Oppiaine —Läroämne — Subject		
Food Sciences (Bioprocessing)		
Työn laji — Arbetets art — Level	Aika — Datum — Month and year	Sivumäärä — Sidoantal — Number of pages
M. Sc. Thesis	December 2020	75

Tiivistelmä — Referat — Abstract

The literature review highlighted the principles of microencapsulation and spray drying, with emphasis on the properties of highly oxidizing oils, protein interface modification, and mechanism of lipid oxidation. The major aim of the research was to study the chemical and physical stability of microencapsulated flaxseed oil (MFSO) during storage under controlled relative humidity conditions (0%, 11%, 33%, 54%, and 75%RHs) and compare the results to bulk flaxseed oil (FSO).

Microencapsulation was done by spray drying of non-cross linked (NCL) and transglutaminase cross-linked (CL) Na-caseinate FSO emulsions. Oxidative stability was examined through changes in physical and chemical properties of MFSOs and FSO as influenced by different RHs and storage periods. Chemical analyses were used to analyze the peroxide value (PV), fatty acid profile, γ-tocopherol, carotenoid and chlorophyll, phenolic compounds, and secondary oxidation products (hexanal and propanal) in MFSOs and FSO. Degree of secondary oxidation was determined by static headspace gas chromatography while the morphology of MFSOs was examined by scanning electron microscopy.

Results showed that at dry condition (0%RH), surface lipids of CL and NCL MFSOs were unstable and more susceptible to oxidation after 17 weeks storage. Release of hexanal and propanal were higher for NCL than CL microencapsulated flaxseed oil which suggested that interfacial cross-linking of Na-caseinate was efficient. At high moisture condition (75%RH), MFSOs were oxidatively stable owing to their low PVs and structural transformation from porous structure to agglomerates or sticky form. High amounts of alpha-linolenic acid and γ-tocopherol were detected in FSO and MFSOs, and only minor losses occurred throughout storage period and under different RHs. FSO also contained considerable amounts of carotenoid and phenolic compounds but low in chlorophyll content. Oxidative stability of interface and matrix elucidated that humidity conditions considerably influenced the chemical and physical properties of CL and NCL MFSOs.

Avainsanat — Nyckelord — Keywords

microencapsulated flaxseed oil, oxidative stability, relative humidity, cross-linked, non-cross linked, peroxide value, flaxseed oil

Säilytyspaikka — Förvaringsställe — Where deposited

The Digital Repository of University of Helsinki, Helda

Muita tietoja — Övriga uppgifter — Further information

EKT Series 1972

PREFACE

This thesis was submitted in partial fulfilment of the requirements for master's degree in Food Sciences. The study was carried out from January 2012 to November 2012 at VTT Technical Research Centre of Finland and Food Chemistry Division under Department of Food and Environmental Sciences (currently Department of Food and Nutrition). The study was sponsored by Raision Research Foundation and other expenses were funded by the Hybridi project of the Department.

The author would like to express her heartfelt gratitude to the committee of supervisors and technical support: Anna-Maija Lampi, Vieno Piironen, Riitta Partanen, Annelie Damerau, and Timo Moisio for their constructive criticisms, valuable comments, and unrelenting support. To my academic supervisors and coordinators: Marina Heinonen, Laila Seppä and Tiina Naskali, for their guidance in all study matters and for organizing the seminars.

To the staff of VTT Technical Research Centre of Finland, Food Chemistry and Food Technology Division of Department of Food and Environmental Sciences (Department of Food and Nutrition) for their technical support and kind assistance during the entire period of research.

To my colleagues for sharing their ideas, knowledge, and experiences during my stay in Finland. Indeed, the entire period of study was a pleasurable and worthwhile experience because of your presence and never-ending support.

To my family, (Tatay Roger, Nanay Mercy, Ate Grace, Kuya Bobit, Kuya Cesar, Naomi, and Nathan) relatives, friends turned family, and to my sisko Malou, tatay Harri, and Mikko for their unconditional love, support, prayers, and kind assistance. To my husband and partner in life (Francis) for the financial assistance, love, care, and understanding in many ways. To my two children (Francis and Cheska) who have been my constant source of inspiration and motivation to pursue my dreams.

Finally, to God almighty and His Angels for giving me the necessary wisdom, strength, courage, guidance, love, and divine providence to carry on the challenges and achieve my goals in life.

Helsinki, Finland 2020

Ma. Heidi Vicente Gabucan

LIST OF ABBREVIATIONS

ALA	Alpha-linolenic acid
CL	Cross-linked
CL-MFSO	Cross-linked microencapsulated flaxseed oil
FA	Fatty acid
FAME	Fatty acid methyl esters
FSO	Flaxseed oil
GC	Gas chromatography
HPLC	High performance liquid chromatography
HS-GC	Headspace gas chromatography
MFSO	Microencapsulated flaxseed oil
MUFA	Monounsaturated fatty acid
Na-caseinate	Sodium caseinate
NCL	Non-cross linked
NCL-MFSO	Non-cross linked microencapsulated flaxseed oil
O/W	Oil-in-water
PUFA	Polyunsaturated fatty acid
PV	Peroxide value
RH	Relative humidity
ROS	Reactive oxygen species
SL	Surface lipids
SEM	Scanning electron micrograph
TL	Total lipids

TABLE OF CONTENTS

1 INTRODUCTION

The increasing demand for food with nutritional and therapeutic benefits has led to the development of microencapsulated products to protect the functional components of lipids. Thus, research on food structure is vital in the development of bioactive ingredients that are stable and easy to consume. Microencapsulation using spray drying is one of the various techniques for the protection of natural compounds of plant oils. It provides great benefits in the manufacture of bioactive oils with improved sensory appeal, nutritional value, therapeutic properties, and storage stability (Gharsallaoui et al. 2007; Jafari et al. 2008).

Numerous studies have proved the efficiency of microencapsulation of oils within a polymeric wall material in protecting the shelf life of polyunsaturated fatty acid (PUFA) from the undesirable effects of lipid oxidation (Drusch et al. 2009). The process of microencapsulation is based on the preparation and drying of oil-in-water (o/w) emulsion to obtain a powdery ingredient in which oil droplets are surrounded by a dry continuous interface of proteins and/or carbohydrates matrix. For several years microencapsulation has been utilized to mask the flavor of undesirable materials as well as to transform liquid into a more convenient powder ingredient (Pegg and Shahidi 2007).

In many process and food systems, it is primarily appropriate to carry out microencapsulation by spray drying conditions rather than freeze drying due to lower operating costs (Velasco et al. 2003). The principle of operation in a spray drier starts with atomization of liquid emulsion, dispersion, or solution into small droplets. Liquid emulsion droplets are brought into contact with hot air inside the chamber to initiate moisture evaporation. The final step is the separation of the dried powder from the air in the cyclone. Overall, the advantages of spray dried material include low water activity, good quality, prolonged storage, convenience in handling, and allows protection of bioactive material from adverse reactions (Shahidi et al. 1993; Partanen et al. 2005; Carneiro et al. 2013).

Previous studies have pointed out an improved oxidative stability when protein interface is enzymatically cross-linked (Partanen et al. 2008; Ma et al. 2012). Thus, in the production of bioactive compounds it is important to determine the factors affecting structural stability with respect to exposure to unfavorable conditions (Buchert et al. 2010; Tonon et al. 2011). In combination with proteins, emulsions are used as a model system to demonstrate factors that

affect the protein interface and carbohydrate matrix upon exposure to various conditions during preparation and storage (Adachi et al. 2003; Walstra and Vliet 2008). In the formation of emulsion, protein molecules and aggregates become adsorbed at the surface of the formed oil droplets. To prevent lipid oxidation in o/w emulsions, interfacial engineering can be utilized to adjust the composition and thickness of the interfacial layer that separates the microencapsulated oils from the aqueous phase (Waraho et al. 2011).

Flaxseed oil is unique among other plant oils and its main application as an industrial oil is based on its high unsaturation, but progressively it is consumed as food oil because it is a significant source of alpha-linolenic acid (ALA). Aside from bakery products, flaxseed oil has also been exploited as a functional component in juices, milk and dairy products, muffins, dry pasta products, macaroni, meat products (Goyal et al. 2014) and soup powder (Rubilar et al 2012) . This research offers a wide area of practical and functional benefits of flaxseed oil in terms of health and nutritional significance (sensory appeal, food safety, and technological point of view).

Flaxseed oil was used as a model oil in this research owing to its high ALA content and susceptibility to lipid oxidation leading to rapid quality deterioration. There have been numerous studies about microencapsulated flaxseed oil, however, only few have provided data on its chemical and structural stability under different relative humidity conditions. It is expected that the samples in this study (microencapsulated oils with cross-linked and non-cross linked Na-caseinate, and bulk flaxseed oil) stabilized under different relative humidities (0%, 11%, 33%, 54%, and 75%) may have influenced the oxidative stability of encapsulation interface and matrix. By controlled storage humidity conditions and following the changes in chemical and physical properties, it may be possible to predict oxidative stability of microencapsulated dried emulsions.

The literature review dealt with the principle of microencapsulation and spray drying, composition of highly oxidizing oils, protein interface modification, and mechanism of lipid oxidation. In previous research on development of stable food structures, the effect of cross linking on protein interface particularly Na-caseinate was reported to be effective in protecting bioactive oils (Ma et al. 2012; Damerau et al. 2014). Thus, the main aim of the experimental research was to study chemical and physical stability of microencapsulated flaxseed oil during storage under controlled relative humidity conditions and compare it to that of flaxseed oil.

Microencapsulation was done by spray drying an emulsion consisting of flaxseed oil and Na-caseinate and maltodextrin matrix. Two different Na-caseinate materials were used: one with native Na-caseinate and one with cross-linked Na-caseinate. Specifically the experimental study aims were to: (1) prepare non-cross linked and enzymatic cross-linked microencapsulated flaxseed oil using spray drying method; (2) analyze the physical and chemical properties of microencapsulated flaxseed oil as a function of relative humidity; and (3) determine the oxidative stability during storage under controlled relative humidity conditions.

2 LITERATURE REVIEW

2.1 Principles of microencapsulation

Research on natural and functional ingredients is essential in the food industry as consumers are becoming increasingly aware about the health effects of the food they consume. Scientific techniques nowadays continue to reveal the benefits of various functional food ingredients such as plant oils. Likewise, different methods have been investigated for the preservation and protection of these natural oils, one of which is microencapsulation.

Microencapsulation is a preservation method of enclosing a sensitive substance in a protective and stable layer which is a capsule, shell, coating, carrier, matrix, or membrane (Jafari et al. 2008). This approach has been useful in many food applications, cosmetic industry, and pharmaceutical where capsules have been perceived as a convenient way of taking medicine (Gharsallaoui et al. 2007; Pegg and Shahidi 2007; Goyal et al. 2014). The microcapsules may vary in diameters from less than 1 µm to 800 µm while the shapes may be different according to the type of materials and process of preparation. The matrix serves to protect the bioactive properties of the core substances during handling, storage, delivery, or various processing. In most cases, dried microencapsulated oils are manufactured by spray drying to produce a powdered food ingredient from natural or formulated emulsion (Figure 1) (Velasco et al. 2003; Gouin 2004; Gharsallaoui et al. 2007).

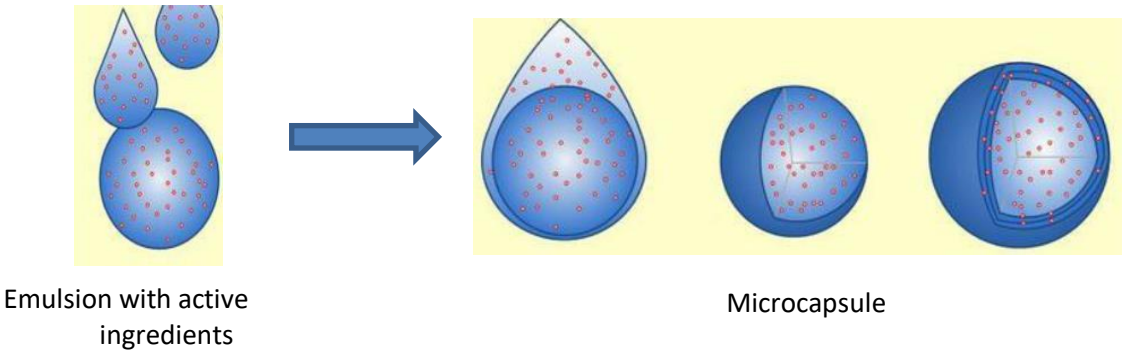


Figure 1. Microencapsulation of bioactive ingredient.

Several purposes have been reported for microencapsulation like protection of functional ingredients from the damaging effects of chemical reactions and external factors like oxygen, temperature, humidity, and UV radiation (Gharsallaoui et al. 2007) enhance the storage and handling properties of a bioactive substance; inhibit the release and loss of highly volatile substances through evaporation; control the rate of diffusion of core particles towards the physical barrier of the matrix; mask the flavor and aroma of dispersed material; and isolate substances in a mixture that has the ability to become reactive with each other (Shahidi et al. 1993; Gharsallaoui et al. 2007; Pegg and Shahidi 2007).

The choice of a suitable wall material as encapsulation matrix is a critical factor in encapsulating functional ingredients. The properties of the matrix have significant effect on protection of functional characteristics of the bioactive substances. A suitable wall material must have good rheological properties; produce stable emulsion; be nonreactive to the bioactive substance during encapsulation and extended storage; provide barrier and protection against environmental factors; offer good solubility in water and ethanol; conform to the standard specifications and properties of solubility of capsules and release of sensitive material from the capsule; be economical and cost effective when utilized on an industrial scale (Pegg and Shahidi 2007). According to Partanen et al. (2008), proteins are usually applied as emulsifying agent in dried emulsions.

Studies have proved the efficiency of microencapsulation of oils within a polymeric wall material while extending the shelf life of PUFAs and protecting it from the adverse effects of lipid oxidation (Frankel 1998; Drusch et al. 2009; Tonon et al. 2011). The microencapsulation process involves the conversion of liquid o/w emulsions into dry powdered ingredients. Many process parameters like emulsification conditions, wall components, and spray drying conditions have been optimized for the microencapsulation (Soottitantawat et al. 2003, Velasco et al. 2003; Pegg and Shahidi 2007).

2.2 Spray drying as a microencapsulation method

Microencapsulation utilizes different techniques and one of the commonly used by food industry is spray drying to preserve flavor and oils from oxidation (Frankel 1998; Gouin 2004; Partanen et al. 2005). Spray drying has been an extensively utilized method of dehydration using hot air

convection drier. The principle of spray drier is based on the transformation of liquid feed solution into powder particles in a constant operation (Shahidi et al. 1993; Gharsallaoui et al. 2007).

Spray drying process mainly involves five steps: (1) concentration of a feed solution, dispersion, emulsion prior to introduction into the spray dryer; (2) atomization of the feed in spray nozzle creating an optimum condition for evaporation of a dried product with the desired properties; (3) atomized liquid is brought into contact with hot air inside the chamber resulting in the loss of moisture in the droplets by evaporation; (4) drying of droplet by two-stage moisture evaporation: the first stage is constant rate of evaporation due to saturated moisture conditions at the droplet surface while the second stage is drying of the wall material due to insufficient moisture to sustain the saturated atmosphere in the chamber; and (5) final separation of powder takes place in the cyclone, filter, and electrostatic precipitators (Vega-Mercado et al. 2001; Vehring 2008). In the evaporation stage, the rate of release of moisture also depends on the diffusion of water through the wall of substances which is increasing in thickness as drying proceeds. A pneumatic hammer is sometimes attached to the chamber to facilitate the discharge of dried powder (Vega-Mercado et al. 2001).

Table 1. Optimum experimental conditions for encapsulation of food ingredients by spray drying method (Jayasundera et al. 2009)

Encapsulated Ingredient	Wall Material	Food Temperature (°C)	Air Inlet temperature (°C)	Air Outlet temperature (°C)
Anhydrous milk fat	Whey protein/ lactose	50	160	80
Ethyl butyrate ethyl caprylate	Whey protein/ lactose	5	160	80
Oregano, citronella, and marjoram flavors	Whey proteins/ milk proteins	NR	185-195	85-95
Soya oil	Sodium caseinate/ carbohydrate	NR	180	95
Calcium citrate calcium lactate	Cellulose derivatives/ Polymethacrylic acid	NR	120-170	91-95
Lycopene	Gelatin/ sucrose	55	190	52
Fish oil	Starch derivatives/ glucose syrup	NR	170	70

Cardamom oleoresin	Gum arabic/ Modified starch/ maltodextrin	NR	176-180	115-125
Bixin	Gum arabic/ Maltodextrin/ sucrose	Room T	180	130
D-Limonene	Gum arabic/ Maltodextrin/ modified starch	NR	200	100-120
L-Menthol	Gum arabic/ Modified starch	NR	180	95-105
Black pepper oleoresin	Gum arabic/ Modified starch	NR	176-190	105-115
Cumin oleoresin	Gum arabic/ Maltodextrin/ modified starch	NR	158-162	115-125
Fish oil	Sugar beet pectin/ glucose syrup	NR	170	70
Caraway essential oil	Milk proteins/Whey proteins/ maltodextrin	NR	175-185	85-95
Short chain fatty acid	Maltodextrin/Gum arabic	NR	180	90

NR – not reported

Jayasundera et al. (2009) reported the suitable wall materials and optimum temperature operating conditions of different ingredients as indicated in Table 1. As shown in the table, combinations of temperature conditions have been commonly used for the microencapsulation of different food materials using spray drying technique. Low molecular weight sugars of wall materials (fructose, glucose, sucrose, and lactose) in the amorphous state have high hygroscopicity and solubility. Fish oil rich in PUFAs was microencapsulated in a matrix of sugar beet pectin and glucose syrup. The authors emphasize the development of substitute natural polymers like gum arabic that could encapsulate flavors like menthol, cumin, black pepper, limonene, and natural color like bixin. Formulation of wall materials included mixtures of different carbohydrates and proteins which were then optimized accordingly. In the microencapsulation of ingredients, high concentrations of about 15–25% gum arabic are generally used for emulsification due to its low protein content (Drusch et al. 2009; Jayasundera et al. (2009).

Some limitations in using the spray drying method can be associated to the sensitivity of components in a solution to high temperature. The method may not be compatible for volatile substances with low boiling point; restricted flexibility in producing particles with complex structures; and extremely sensitive bioactive substances like high temperature sensitive microorganisms, enzymes, and vitamins (Buckton 2002; Pegg and Shahidi 2007). However,

these limitations can be lessened if suitable coating materials are used and drying parameters are controlled and optimized. Spray drying has advantages that can be designed to fit every capacity required for processing. The operating system is continuous and adjustable to complete mechanical control (Buckton 2002; Gharsallaoui et al. 2007). Depending on the capacity of the drier the feed rate usually ranges from a few kilograms per hour to over 100 tons per hour. It can be used with both heat-resistant and heat sensitive products and almost spherical particles can be formed (Gauvin and Katta 1976).

2.3 Properties of the protein interface

Food structure can be modified in several ways by addition of hydrocolloid, emulsifiers, stabilizers, and thickeners or through structural transformation by covalent cross-linking between food biopolymers including proteins and carbohydrates (Buchert et al. 2010). Protein interface engineering continues to be an interesting field of research due to its biological activity. Since protein molecules can be easily controlled, protein cross-linking and modification are frequently utilized to determine the functions of each amino acid side chains in the physical, chemical, and biological properties of proteins. In the production of functional food ingredients, it is important to examine the harmful effect of exposure to interfaces on the structural stability of proteins in emulsions (Dickinson 1997; Buchert et al. 2010; Tonon et al. 2011).

Proteins are complex macromolecules which play an essential role in the structure and function of most foods. Proteins are composed of amino acids arranged in a linear chain and attached together by peptide bonds between the carboxyl and amino groups of adjacent amino acid residues (Belitz et al, 2004; Coultate, 2009). Milk proteins in soluble and dispersed form are widely used as food ingredients with good surface active and colloidal stabilizing characteristics (Dickinson 1997).

During emulsion preparation, various protein molecules and aggregates become rapidly adsorbed at the surface of the newly formed oil droplets. For oil in water emulsion made with milk protein as emulsifier, the initial droplet size distribution is an important factor influencing the emulsion shelf-life (Walstra and Vliet 2008; Tonon et al. 2011). At pH below the protein iso-electric point, protein interfaces have exhibited to slow down lipid oxidation (Moisio et al. 2014). As reported, there was improved thickness and cohesiveness of interfacial protein layer

when cross-linking was carried out before emulsification process and prevented lipid oxidation in emulsions (Ma et al. 2012).

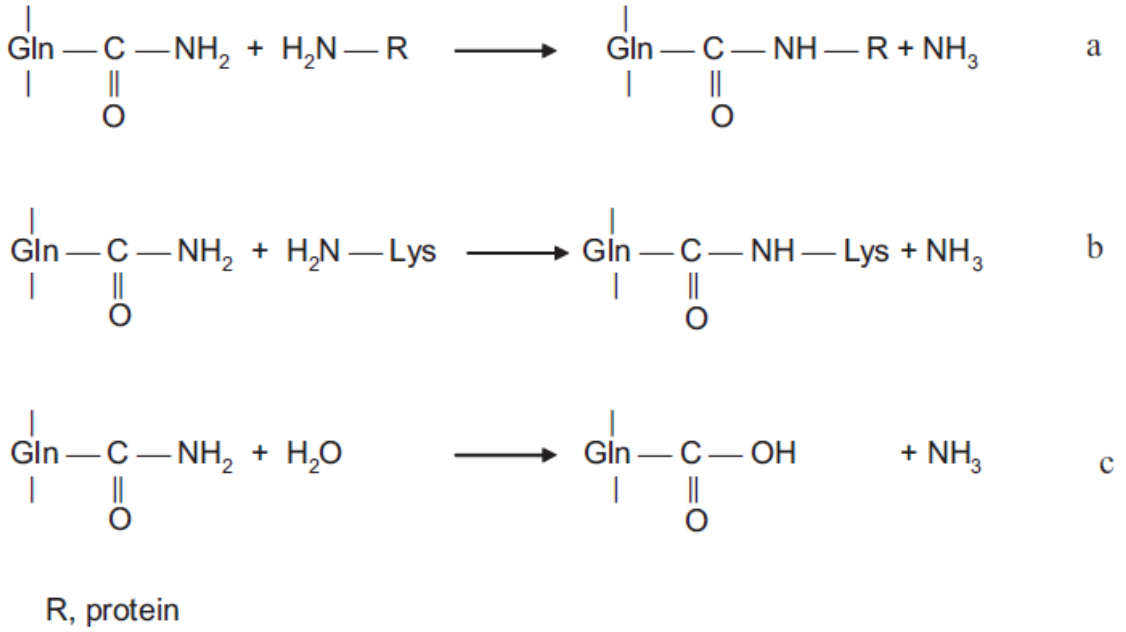
There are various mechanisms of formation of multi-component microcapsules which begins with the formation of a multiple component solution or emulsion. Most researchers focus on the investigation of the effect of emulsion formation in the process of encapsulation. Water-in-oil emulsions are flexible formulations, and the release of incorporated hydrophilic molecules of various sizes and characteristics can be modified by adjusting parameters such as volume fraction of the dispersed phase, droplet size and osmotic gradient. In combination with proteins, emulsions are used as a model system to demonstrate the interfaces and pressures that proteins are exposed to during preparation (Adachi et al. 2003; Walstra and Vliet 2008).

The preparation and formation of emulsion includes a combination of two phases, an interface and application of various types of mechanical pressure. During emulsion preparation, the protein is exposed to mechanical stress from the homogenization as well as to the components in the emulsion. The protein is exposed to the mixing of the oil and aqueous phase, hence, the formation of the oil-water interface (Adachi et al. 2003). Proteins are amphipathic molecules, and they have been used for their surfactant properties in food science to stabilize oil-in-water emulsions (Belitz et al, 2004; Coultate, 2009). This also makes them susceptible to structural changes when exposed to the interfaces created during the preparation of the particulate system (Walstra and Vliet 2008).

2.4 Enzymatic modification of the protein interface

Enzymatic cross-linking has also been studied using the enzyme transglutaminase (EC 2.3.2.13). This transglutaminase belongs to the group of acyltransferases that induce an acyl transfer reaction using peptide bound glutamine residues as donors and several primary amines like peptide bound lysine as acyl acceptors (Traore and Meunier 1992; Sakamoto et al. 1994). Various substrates including the ε-amino group of lysine or lysyl residues in proteins will result in polymerization or amine incorporation (Figure 2 a,b) (Traore and Meunier 1992). The enzyme can form intra- or intermolecular ε – (γ-glutamyl) lysine isopeptide bond. During this reaction, one molecule of ammonia is released per crosslink. When the amine substrates are not available as acyl acceptors, transglutaminase can catalyze deamination of glutamyl residues using water

as an acyl acceptor (Figure 2c). Transglutaminases have a wide specificity for the acyl acceptor substrates, in contrast to their limited glutamine (an acyl donor) substrate specificity. Several milk proteins like caseins are reported as good substrates for transglutaminase catalyzed crosslinking (Sakamoto et al. 1994; Faergemand et al. 1998).



where: Gln - glutamyl residues
Lys - lysine or lysyl residues

Figure 2. Transglutaminase catalyzed reactions (a) cross linking via a lysyl residue (b) incorporation of Ɛ-amino group of lysine and (c) deamidation. (Adapted from Buchert et al. 2010)

Protein modification by transglutaminase may be exploited in a number of applications in the food industry such as by enhancing rheological properties, improving gel formation and gel properties, modification of protein solubility, foaming properties, and water-holding properties (Motoki and Seguro 1998; Zhu et al. 1999, Jaros et al. 2006b). Protein cross-linking by enzymatic technique suggests numerous potential benefits through specific and natural ways of converting food structure. The sequence of reaction mechanisms of different types of enzymes may vary which results in different technological properties. The existing commercial applications are based on transglutaminase. Knowledge of the chemical reactions, as well as the technological and physiological impact of the enzyme stages, is important when these new enzyme concepts are being implemented. Research on other types of cross-linking enzymes can

be exploited such that new enzymes might be introduced into the market and food industry (Motoki and Seguro 1998; Zhu et al. 1999).

Protein enzymatic cross-linking is also dependent on the main factors such as optimum activity and stability conditions like temperature, pH, and inhibitors, and the morphological state of substrate molecule at the reaction conditions. Several studies showed that globular proteins are more available to enzyme active sites rather than nonglobular proteins. As an example of a good substrate for transglutaminase are caseins due to their flexibility and open tertiary structure (Buchert et al. 2010).

Research on the phenomena behind the effect of protein interface modification by transglutaminase enzyme is important in spray dried emulsion system. Results generated from previous studies point out the benefit of enzymatic cross linking in improving oxidative stability of protein interface (Partanen et al. 2008). In a research by Partanen et al. (2008), the whey protein isolate as matrix inhibited flaxseed oil oxidation as compared to the bulk oil. The rate of lipid oxidation in flaxseed oil with the matrix was highest at 0% and high at 91% relative humidity. The difference in the present study with that of Partanen et al. (2008) was that combinations of protein (Na-caseinate) as interface and carbohydrate (maltodextrin) as matrix were utilized and their chemical and physical properties were examined as influenced by various humidity conditions and storage periods.

In a study by Partanen et. al. (2008) on oxidative stability of flaxseed oil, they reported that moisture influenced changes in the matrix and controlled the rate of oxygen diffusion into the surface. In the same case, the rate of hydroperoxide formation as the reactive oxygen consuming step was also influenced by moisture. The study investigated the difference in protein-based matrices. For globular protein matrices the oxygen is rate-limiting by the thick interfacial layer between the oil and bulk protein phases. But this also depends on water and the composition of the bulk matrix.

Competitive adsorption is a common characteristic of many systems containing a mixture of surface-active group such as proteins. Competitive surface adsorption between surface-active substances in liquid formulations can be employed to better encapsulate and protect a sensitive and bioactive protein or enzyme formulation and to modify the powder properties. Surface

competition during spray drying involves adsorption of surface-active components to the air or liquid interface of drying droplets (Elversson and Millqvist-Fureby 2005).

2.5 Chemical composition of flaxseed oil and comparison to other vegetable oils

Oxidation of polyunsaturated oils have posed a major problem in the food industry owing to the negative effects on nutritional value, flavor, safety, and storage because of adverse oxidation reactions. PUFAs are easily oxidizing lipids due to their sensitivity upon exposure to light, air, heat, and metals, which upon exposure may produce undesirable volatile substances causing problems in food products. The most common sources of PUFAs are marine or fish oils and plant oils. The sensitivity of PUFAs is due to their chemical structure because they have bis-allylic double bonds, which are easily oxidized. The saturated fats oxidize more slowly because they have no double bonds (Mc Clements and Decker 2008).

Flaxseed is one of the most important oilseed crops used for many industrial and nutritional purposes. Every part of the flaxseed plant is used commercially, either directly or after processing. Among the exceptional values of flaxseed oil is a high content of ALA, plant lignans and dietary soluble fiber. Flaxseed oil is not normally consumed as food oil because it has extremely low oxidative stability. Due to high unsaturation degree, flaxseed oil may easily oxidize during processing, handling, and storage. Its main application as industrial oil is also based on its instability, but increasingly it is consumed as food oil due to its contribution as a functional food. As a dietary supplement, flaxseed oil is often sold in capsule form or liquid stored in bottles. As for its usage, different brands of flaxseed oil are now sold in health food stores as a healthy option and substitute for fish oil (Cunane and Thompson 1995; Herchi et al 2011; Tonon et al. 2011; Yu et al. 2017).

The oil is extracted from seeds of flaxseed plant (*Linum usitatissimim*) and considered as a dietary source of ALA which represents 45-60% of its total fatty acids (Vaisey-Genser 1994; Tarpila et al. 2005; Carneiro et al. 2013). The ground or whole flaxseeds are edible and have found many applications in many bakery and confectionery products to improve dietary value of a food by providing a good source of essential omega-3 fatty acid, ALA (Tonon et al. 2012).

The whole flaxseeds contain about 25% fiber, of which 20–40% is soluble fiber (Vaisey-Genser 1994) which performs a key function in lowering plasma cholesterol. Flaxseed is an abundant source of plant lignans, a potential antioxidant recognized to have defense and protective factor against neurological disorders, hormonal imbalances as well as related cancers of the breast, prostate and colon (Carter 1993; Vaisey-Genser 1994; Herchi et al 2012). Several researchers have investigated the health benefits and therapeutic aspects of flaxseed and flaxseed oil with particular importance on their high fiber and omega-3 fatty acid contents, and their potential application in bakery products and other food items (Carter 1993; Herchi et al 2012).

Tables 2 and 3 show the fatty acid composition of flaxseed and other oils. In flaxseed oil, the most abundant fatty acid was ALA (52%) followed by oleic acid (20%), linoleic acid (17%), palmitic acid (7%), and a lower content of stearic acid (4%). The P/S ratio was in the average for flaxseed oil (6.3) while safflower oil had the highest value of 8.8. Fatty acid composition may also vary in oilseeds, but flaxseed oil with high ALA varieties may contain up to 60% of ALA. As shown in Table 3, significant concentration of γ-tocopherol and traced amounts of δ-tocopherol and α-tocopherol were found in flaxseed oil (430-575 mg/kg). Tocols provide an antioxidant activity in many plant oils like flaxseed oil in protecting the monounsaturated fatty acids (MUFAS) and PUFAs from oxidation, which may also explain the high concentration of these phenolic antioxidants in highly unsaturated edible plant oils (Shahidi and de Camargo 2016). The lipid fraction of plant edible oils is the most important natural dietary source of tocopherols and tocotrienols, although tocotrienols were not found in flaxseed oil (Schwartz et al. 2008). In flaxseed oil, lipids are protected against oxidation also by the presence of other antioxidants such as phenolic acids and carotenoids. Carotenoids are considered to play an important role as free radical scavengers by electron transfer to their double-bond structure (Hasiewicz-Derkacz et al. 2015). The scavenging ability of phenolic acids is based on their electron-donating ability and inhibition of lipid peroxidation induced by superoxide. Tocopherols typically block the hydroperoxide intermediates and prevent the autoxidation chain reaction whereas carotenoids perform as scavenger of singlet oxygen (Tuberoso et al. 2007; Hasiewicz-Derkacz et al. 2015).

Table 2. Fatty Acid composition of linola, traditional flaxseed and other oils (Haumann 1990; Green and Dribnenki 1994).

Oilseed	Fatty Acid (%wt)					
	16:0	18:0	18:1	18:2	18:3	P/S Ratio
Linola	6	4	16	72	2	7.4
Safflower	7	2	12	79	-	8.8
Sunflower	7	4	16	73	-	6.6
Corn	11	2	27	59	1	4.6
Soybean	12	4	25	51	8	3.7
Canola	5	2	66	19	8	3.9
Flax	7	4	20	17	52	6.3

P/S ratio- Polyunsaturated/saturated fat ratio

Table 3. Characteristics and fatty acid composition of high linolenic acid flaxseed oil varieties (Gunstone 1996)

Parameter	**Values**	
Specific gravity (20ºC)	0.927-0.932	
Refractive index(20ºC)	1.478-1.482	
Iodine value	170-203	
Saponification value	188-196	
Unsaponifiable matter (%)	1.5 max	
Fatty Acid Composition (%wt)	**Typical**	**Range**
14:0	tr	tr
16:0	6.0	5-7
16:1	0.1	tr – 0.2
18:0	2.5	2-6
18:1	19.0	14-40
18:2	24.1	14-19
18:3	47.4	35-60
20:0	0.5	0.1-0.7
Others	0.4	1.0 max
Tocopherols (mg/kg)	**Values (mg/kg)**	
α - tocopherol	5-10	
γ - tocopherol	430-575	
δ - tocopherol	4-8	
Total	440-588	

2.6 Mechanism of lipid oxidation

Lipid oxidation of polyunsaturated fatty acids leads to the development of many lipid oxidation products, including e.g. volatile secondary oxidation products (Figure 3). The mechanism of lipid oxidation has three phases: initiation, propagation, and termination (Figure 3). Lipid oxidation is initiated by free radicals and propagates by a series of autocatalytic free radical chain reactions (Frankel 1998; McClements and Decker 2008; Coultate 2009). The first reaction is an attack of a free radical ($X^{\bullet}$) on a lipid molecule (R) (Figure 3). When initial radicals are formed, the development of other radicals proceeds rapidly. Free radicals are unstable, and they look for a partner for their unpaired electron. During propagation reactions, the lipid radical ($R^{\bullet}$) reacts with atmospheric oxygen to further produce peroxyl radicals ($ROO^{\bullet}$). These are extremely reactive compounds that also react with unsaturated fatty acids by abstracting a hydrogen atom to create hydroperoxides (ROOH) and reproduce again a new lipid radical $R^{\bullet}$. The reaction of lipid radical with oxygen is repeated, and peroxyl radicals and hydroperoxides are formed. In addition, when present at high levels hydroperoxides start to break down to form new radicals such as $R^{\bullet}$, $ROO^{\bullet}$ and alkoxyl radicals ($RO^{\bullet}$). In the propagation reactions, there is accumulation of increasing quantity of free radicals in the unsaturated fat which consequently take in substantial amount of oxygen from the atmosphere. When the number of free radicals reaches at a maximum point, peroxyl radicals react with one another and form stable non-propagating end-products (Frankel 1998; McClements and Decker 2008; Coultate 2009).

Initiation	$X^{\bullet} + RH \longrightarrow R^{\bullet} + XH$
	$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$
Propagation	$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$
	$2ROOH \longrightarrow RO^{\bullet} + ROO^{\bullet} + H_2O$
Termination	$R^{\bullet}, RO^{\bullet}, ROO^{\bullet} \longrightarrow$ stable, non-propagating species

where: $X^{\bullet}$ - free radical; R – lipid molecule; $R^{\bullet}$ - lipid radical; $RO^{\bullet}$ - alkoxyl radicals; $ROO^{\bullet}$ - peroxyl radicals; ROOH – hydroperoxides

Figure 3. Steps in the autoxidation of unsaturated fatty acids.

Lipid oxidation of microencapsulated oils is affected by microcapsules characteristics. Drusch et al. (2009) investigated the effect of spray-drying conditions on particle characteristics and oxidation of microencapsulated fish oil and showed that high inlet and outlet air temperature may lead to autoxidation of the encapsulated and non-encapsulated core material already during the drying process, if a wall material with strong film forming properties is used.

2.7 Measurement of lipid oxidation in microencapsulated foods

Food lipids are likely to have high levels of lipid oxidation products due to the extraction and refining processes they undergo like for fats and oils. Figure 4 illustrated the changes and effects of lipid oxidation products on product quality. Lipid oxidation in microencapsulated foods is usually measured by formation of primary oxidation products (hydroperoxides) and secondary oxidation products (aldehydes, ketones, hydrocarbons, acids, and epoxides). The development of hydroperoxides from PUFA may possibly result in double bond isomerization. In addition to peroxide value (PV), the determination of conjugated dienoic and trienoic fatty acid derivatives allow characterization of oxidation in food products. PV measurement (by iodine titration or ferric thiocyanate method) for microencapsulated foods is a useful method for monitoring oxidative deterioration, and it is usually combined with another method of examining secondary oxidation products to provide clear results of the progress of oxidation (Waraho et al. 2011; Vieira et al. 2017).

Lipid oxidation can cause undesirable aromas and flavors known as rancidity (Figure 4). During lipid oxidation structural and textural changes may occur in protein cross-linking; and the loss and co-oxidation of nutrients and pigments such as vitamin A, vitamin E (tocopherols), vitamin C, and flavonoids (Vieira et al. 2017). Certain losses of important nutrients may also render microencapsulated foods unfit for consumption (Hasiewicz-Derkacz et al. 2015; Vieira et al. 2017). Food emulsions are also susceptible to oxidation due to their high surface area that creates lipid-water interfaces where lipid and prooxidants can readily interact (Hasiewicz-Derkacz et al. 2015). Likewise, darkening of color (Figure 4) is caused by the formation of melanoidins from proteins alone or from carbohydrates and proteins, and loss of color due to free radical destruction of conjugated double bonds like in carotenoid bleaching (Vieira et al. 2017).

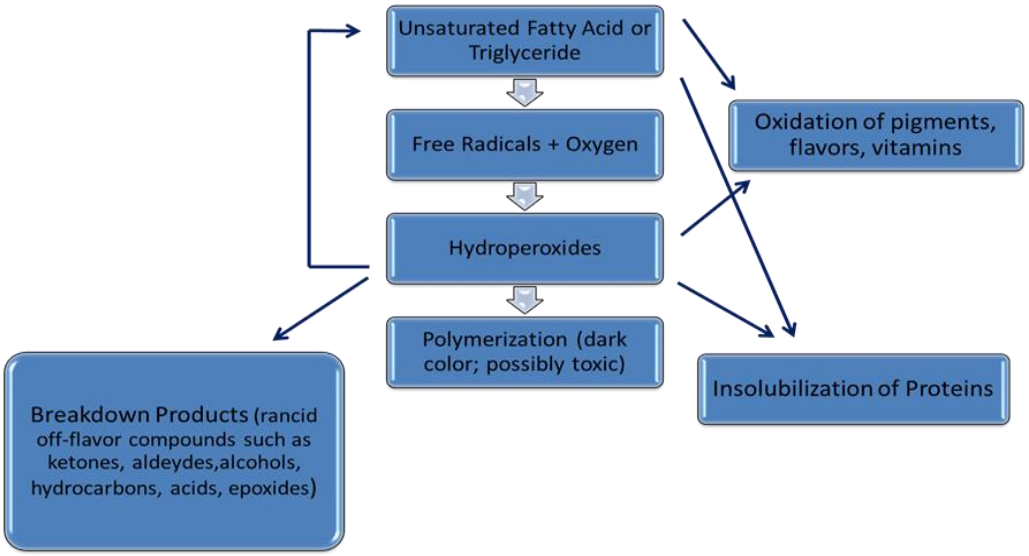


Figure 4. Lipid oxidation products and their effects on the quality of the products.

Another method to examine the release of secondary products of oxidation is through static headspace-gas chromatography. The hydroperoxide decomposition leads to generation of aldehydes such as propanal and hexanal (Frankel 1998; McClements and Decker 2008). Static headspace-gas chromatography (HS-GC) is an essential separation method for studying volatile organic compounds over a wide range of sample matrices. In HS-GC, a small amount of sample is placed into a vial, and vials are heated at a certain temperature and time combination until equilibrium is reached between the non-volatile sample matrix and volatile compounds. Thereafter, the volatile compounds are injected into a GC, where they are separated from each other by passing through the stationary column with the carrier gas. Flame-Ionization-Detector (FID) detects the eluting compounds, and the results are shown as a chromatogram with peak areas and retention times. Retention times are associated to the time between the analyte is introduced into the column and is detected, while the peak areas (or height) are related to the quantity (Christie 1989; Kolb, 1997). Volatile aldehydes are identified based on their retention times.

3 EXPERIMENTAL RESEARCH

3.1 Background and aims

Flaxseed oil is recognized as an important oilseed with a lot of nutritional benefits. It contains a significant quantity (45-60%) of omega-3 fatty acids or PUFA which makes it susceptible to oxidation reactions. Microencapsulation using spray drying is utilized to protect the bioactive components in flaxseed oil and make it more convenient to consume. During storage and exposure to different relative humidities, the structure of microencapsulated powder may change and may have an effect on lipid oxidation. Hence this study was realized to follow the behavior of three types of samples: (1) microencapsulated flaxseed oil with cross-linked sodium caseinate and (2) microencapsulated flaxseed oil with non-cross-linked sodium caseinate (3) and bulk flaxseed oil.

To reach the aims of the study, chemical and physical stability of microencapsulated flaxseed oil and the chemical stability of flaxseed oil were studied during a storage experiment at various humidity conditions (0%, 11%, 33%, 54%, and 75%RHs) and different timepoints (1, 3, 5, 9, 13, and 17 weeks). The flaxseed oil and microencapsulated flaxseed oil were stabilized and stored at varying levels of relative humidities. After each storage times, chemical characterization was carried out to measure the peroxide value (PV), fatty acid methyl esters (FAME), tocopherol content, hexanal and propanal content from bulk flaxseed oil and microencapsulated flaxseed oils. Physical characterization was done by examining the morphological structures of microencapsulated flaxseed oils and determining how storage time and conditions affected the spray dried flaxseed oils.

3.2 Materials and methods

3.2.1 Materials

The commercial flaxseed oil utilized as raw material in the study was obtained from Elixi Oil Oy (Somero, Finland). Sodium caseinate from Kaslink Foods (Koria, Finland) contained a minimum of 90% protein, a maximum of 1.5% fat, 4.0% ash, and 5.5% moisture. Maltodextrin

with a dextrose equivalent (DE) of 22.2 was purchased from Grain Processing Corporation (Muscatine, Iowa, USA). Microbial transglutaminase enzyme Activa MP was obtained from Ajinomoto (Japan) and was further purified and evaluated for activity.

3.2.2 Standard and reference materials

During fatty acid determination, a methyl ester C19:0 was used as an internal standard while a mixture of GLC-63 (Nu-Check-Prep, Elysian, MN, USA) was obtained as a reference standard to identify the fatty acid methyl esters. A small amount of flaxseed oil from Elixi Oil Oy (Somero, Finland) was utilized as a reference and sample material for fatty acid analysis.

3.2.3 Reagents for chemical analysis

The reagents used in the extraction of total lipids were heptane (C_7H_{16}) and isopropanol in HPLC grade from Rathburn Chemicals (Walkerburn, Scotland).

In the fatty acid methyl ester determination, reagents utilized were sodium hydroxide (NaOH) (Merck, Darmstadt, Germany), boron trifluoride-methanol solution (BF_3CH_3OH) (Sigma-Aldrich, Steinheim, Germany), and a drying agent with 99% purity sodium sulfate (Na_2SO_4) (VWR International, West Chester, PA, USA).

In peroxide measurement, the solvent mixture contained HPLC grade methanol (CH_3OH) (Rathburn Chemical, Walkerburn, Scotland), decanol (Merck, Hohenbrunn, Germany), and heptane. Other chemicals used were iron (II) chloride tetrahydrate ($Fe(II)Cl_2 \cdot 4\ H_2O$) from Sigma-Aldrich (Steinheim, Germany), iron (III) chloride ($Fe(III)Cl_3$ from Merck (Darmstadt, Germany), and ammonium thiocyanate (NH_4SCN) from Sigma-Aldrich (Steinheim, Germany).

For storage stability test, five different saturated salt solutions were prepared to attain the appropriate relative humidity conditions in each desiccator. The salts were purchased from Sigma-Aldrich (Steinheim, Germany) and they were of 98% purity: dry phosphorus pentoxide (P_5O_{10}), lithium chloride (LiCl), magnesium chloride hexahydrate ($MgCl_2 \cdot 6\ H_2O$), magnesium nitrate hexahydrate ($Mg(NO_3)_2 \cdot 6\ H_2O$) and sodium chloride (NaCl). Phosphorous pentoxide

was used as such. From all other salts, saturated solutions were prepared using purified Milli-Q water (Millipore Corp., Bedford, MA, USA).

For tocopherol analysis, solvents used were HPLC grade 1,4 dioxane (Riedel-de Häen, Seelze, Germany), Milli-Q water, (Millipore Corp., Bedford, MA, USA), 99.5% ethanol (ALTIA, Rajamäki, Finland), heptane and 2-propanol (Rathburn Chemicals Ltd., Walkerburn, Scotland)

For the determination of total phenolic compounds, solvents used were HPLC grade heptane (C_7H_{16}), methanol (CH_3OH**)**, and ethanol from Rathburn Chemicals (Walkerburn, Scotland).

3.2.4 Preparation and spray drying of flaxseed oil emulsion

Samples for cross-linked and non-cross linked microencapsulated flaxseed oil were prepared starting with sodium caseinate solution, usually prepared 1 day prior to spray drying. Sodium caseinate powder (0.5-3.0% w/w) was dissolved in boiling water. The solution was mixed with magnetic stirrer for 30 minutes at 1000 rpm while adding sodium azide (0.04% of protein solution) to prevent microbial growth during overnight stirring. In a separate 1 L glass beaker, a mixture of maltodextrin (DE = 22) was dissolved in Milli Q-water (50% solution) and stirred for about 2-3 hours. The maltodextrin solution was then adjusted to pH 6.5 by adding ~40 drops of 1 M NaOH.

When preparing the emulsion, 600 g of sodium caseinate solution was weighed in a 1 L Schott bottle beaker and 150 g flaxseed oil was added. The emulsion was pre-homogenized twice in a Heidolph DX 900 homogenizer at speed 6 for 2 minutes. A second homogenization was performed in 10 cycles using a Microfluidics 110Y homogenizer set at 40 psig/press and 500 bar, making sure that the interaction chamber (75 µ) was attached first and parallel to the auxiliary chamber (200 µ).

In a separate 1 L Schott bottle, 535 g of the previously homogenized emulsion containing (80% protein solution and 20% flaxseed oil) was mixed with 500 g of 50% maltodextrin solution. The mixture was stirred with magnetic bar in a Heidolph MR2002 mixer for 3 minutes at 1000 rpm. The emulsion was dried in a NIRO A/S MOBILE MINOR™ '2000' (Niro A/S, Soeberg, Denmark) spray dryer. The equipment was adjusted according to previously optimized conditions using inlet process gas temperature (180^0C), outlet process gas temperature (80^0C $\pm$

2^0C), atomization pressure (4 bars), peristaltic pump (3-5 in pump scale), and pneumatic hammer pressure of 2 bars. The atomizer rotating speed was set to 22000 rpm. After drying was completed, samples were collected from glass vessel attached to the chamber and cyclone of the spray dryer. The final dry matter composition of the spray dried emulsion was 30% flaxseed oil, 67% maltodextrin, and 3% sodium caseinate.

For the cross-linked sample, the same procedure was followed as with non-cross linked but with the addition of 250 µl of transglutaminase (P482) enzyme to the sodium caseinate solution. The transglutaminase enzyme was used to cross link the Na-caseinate. Analytical grade sodium hydroxide (1 M) was added to the emulsion to adjust the pH, while sodium azide was mixed to preserve the emulsion from microbial contamination. Incubation of the caseinate solution with enzyme was carried out for 4 hours by continuous mixing with a magnetic bar, added with maltodextrin solution and then spray dried.

The spray dried emulsions were prepared according to the procedure by Moisio et al. (2014) containing the following materials: flaxseed oil (30% dry matter), Na-caseinate in cross linked or non-cross linked form (3% dry matter), maltodextrin (67% dry matter).

3.2.5 Stabilization of bulk flaxseed oil and microencapsulated flaxseed oil at different relative humidities

For measurement of hexanal and propanal, approximately 0.5000 to 0.5099 g of each microencapsulated flaxseed powder and oil were weighed in clean headspace vials in triplicate for each time point and relative humidity. For other chemical analyses, ca. 3 g of each material was weighed in clean headspace vials once for each time point and relative humidity. The vials were arranged and closed in storage desiccator set at different relative humidities (0%, 11%, 33%, 54%, and 75%RHs) and stored according to different storage periods (1, 3, 5, 9, 13, and 17 weeks). The relative humidity was maintained using various types of saturated salt solutions.

3.2.6 Extraction of surface and total lipids

Extractions of surface and total lipids were done as presented earlier by Damerau et al. (2014). From the previously mixed sample, approximately 0.3000 to 0.3099 g was weighed using analytical balance (Precissa XT 220A) in 30 mL extraction glass tubes. Weighing was done in triplicate both for the surface and total oil extraction. Extractions were done in the dark room by shaking the tubes with fitted covers in a shaker (Desaga Heidelberg Nr. 782031) at constant motion for 15 minutes. After shaking, tubes were taken out and placed in the centrifuge (Hermle Model Z323) at 3000 rpm and brake 6 for two minutes. The organic phase that has separated was decanted using glass pasteur pipette into 10 mL kimax tubes.

For the surface oil extraction, 5 mL of heptane was added into the tubes containing the powder. For total lipids extraction, 3 mL of Milli Q water heated in water bath at 40^0C was pipetted into each of the glass tubes to wet the powder. To make sure that there were no visible powder particles, the solution was agitated in a vortex mixer at 3000 rpm for 1 minute. Another 10 mL of a mixture of heptane and isopropanol solvent (3:1 v/v) was placed into the tubes containing the wetted powder samples.

The extracted oil from the microencapsulated samples was divided according to analytical measurements: 1 mL of the oil extract was used for the fatty acid methyl ester (FAME) analysis, 1.5 mL was utilized for tocopherol analysis, and 150 µl was taken for PV measurement.

3.2.7 Peroxide measurement

Ferric thiocyanate method by Lehtonen et al. (2011) was used to measure the hydroperoxides as primary oxidation products. For preparation of calibration curve, seven test tubes with fitted stoppers were prepared according to Table 4. Each of the two solutions ($FeCl_3$ and HCl) were added to each test tube. Using a pipette, 9.8 mL of the solvent mixture and 50 µL of NH_4SCN were added into each tube. Timing was set at least 20 seconds when adding in NH_4SCN in between the standards. The standard was measured in spectrophotometer at 500 nm (Lambda 25 UV/VIS, Perkin Elmer Inc., Waltham, MA, USA) after 5 minutes. Calibration of the instrument was done before analyzing the sample extracts. Calibration curves were obtained

for every peroxide measurement, and correlation coefficients (R^2) were between 0.990 to 0.9990.

Table 4. Standard line preparation for calibration of peroxide measurement.

Fe (III), µg	$FeCl_3$, µL	3.5% HCL, µL
0	0	200
5	25	175
10	50	150
20	100	100
30	150	50
40	200	0

For the analysis of extracts, the following mixtures were combined in test tubes and performed in duplicate: 150 µL mL of the extracted samples from surface or total lipids, 50 µL blank (heptane), 9.8 mL solvent, and 50 µL NH_4SCN. In each tubes 50 µL of $Fe(II)Cl_2$ was pipetted every 20 seconds in between samples. Measurement of absorbance was done in spectrophotometer at a wavelength of 500 nm after five minutes. Results were calculated according to standard curve and absorbance obtained using the equation by Hornero-Mendez et al. (2001).

$$PV\ \left(\frac{mekv}{kg}\right) = \frac{Asm - Abl - s}{55.84\ x\ 2\ x\ b\ x\ Lsm}$$

where: Asm = absorbance of sample
Abl = absorbance of blank
s = slope of Fe(III) calibration curve
b = y intercept of Fe(III) calibration curve
55.84 = molecular weight of Fe
2 = correction factor to convert mekv of Fe to mekv of peroxide
Lsm = lipid content of the sample

3.2.8 Fatty acid determination of bulk oil and spray-dried emulsions

The fatty acids from the extracted oil for surface and total lipids were methylated and analyzed as methyl esters using gas chromatography. Fatty acid determination was based on the protocol by Soupas et al. (2005) and Damerau et al. (2014). A reference sample was prepared by weighing 200 mg flaxseed oil diluted in 10 mL heptane; from this solution 1 mL was taken and

placed in 10 mL kimax tubes. An internal standard (C19:0) of 1 mL (5 mg /ml for oils, 2.5 mg/ml for powders) was added to the reference oil and samples.

Duplicate samples from the surface and total lipid extract were prepared by placing 1.5 mL in kimax tubes followed by adding 0.5 mL internal standard. Both the oil and extracts were evaporated to dryness using nitrogen gas. After evaporation, each tube from oil and extracts were mixed with 1 mL of 0.5 M NaOH in methanol. The mixture of reference and test samples were boiled for 5 minutes and cooled down before adding 2 mL BF_3CH_3OH into each tube. The tubes were immersed again in boiling water for 5 minutes and cooled down for few minutes. After cooling, the tubes were added with 1 mL saturated NaCl solution and 1 mL of heptane to the surface and total lipid extract then 4 mL to the reference flaxseed oil mixture. The mixture was mixed in a vortex for 1 minute, after which the phases were allowed to separate. The upper layer containing methyl esters in heptane was transferred into another tube with drying agent (Na_2SO_4) and allowed to stand for 30 minutes after which the aliquot was transferred into the GC vials for capillary gas chromatography analysis.

The fatty acid methyl esters were analyzed using gas chromatography (Series 6890N, Agilent Technologies Inc., Wilmington, DE, USA). The GC was attached with a capillary column NB-351 (Nitroterepthalate modified polyethylene glycol; 25m long, 0.32 i.d., 0.2 μm film thickness; Nordibond Ltd., Finland) and flame ionization detector (FID). Helium was utilized as the carrier gas. The injection temperature was set to 240^0C while the detection temperature was 260^0C. The extract was injected at 1/20 split ratio and the GC computer program was set at 160^0C for 1 minute, and temperature was gradually increased from 4^0C to 240^0C for 5 minutes. Injection volume was 1 μL. Fatty acid methyl esters (FAME) were identified by comparing using a reference standard mixture GLC-63. Quantification was done through comparison of the peak areas of FAME and internal standard.

3.2.9 Tocopherol analysis of bulk oil and spray-dried emulsions

The tocopherol analysis described by Schwartz et al. (2008) and Damerau et al. (2014) was followed. The standard solution for tocopherol measurement was prepared by pipetting 1 mL from 500 mg/L standard of α-, β-, γ-, and δ-tocopherol into the volumetric flask. The solvent was evaporated under nitrogen gas. Heptane was added to volumetric flask and the flask was

filled up to the mark. A concentration of 2 and 0.2 mg/L was transferred to the HPLC vials for analysis.

The surface and total lipid extracts from the cross linked and non-cross linked were measured for tocopherol composition using normal phase HPLC with fluorescence detection. In-house reference and sample oil were prepared from flaxseed oil and rapeseed oil as reference oil. The extracts and oil were evaporated in isopropanol-heptane solvent mixture and then added with heptane. Using a syringe filter, the aliquot from the extract and reference oil solution was transferred to HPLC vials.

The oil extracts, reference oil, and standard solution were then measured in HPLC according to usual conditions. Tocopherols were separated in 20 minutes with a mobile phase which consist of 97% heptane and 3% 1,4 dioxane (v/v) and a flow rate of 2 mL per minute. Column was maintained at 30^0C and the auto sampler tray cooled to 4^0C. Tocopherols was detected by fluorescence detector set to λ_{ex}= 290 nm and λ_{em} = 325 nm). Quantitative analysis was executed using external calibration curve generated by twice of each of the calibration points with concentrations containing 2, 5, 10, 20, 50 and 80 ng/injection.

3.2.10 Hexanal and propanal measurement by static headspace gas chromatography

For hexanal and propanal analysis, static headspace gas chromatography with flame ionization detection was followed according to the methods used by Damerau et al. (2004) and Rey et al. (2005). For each time point (1, 3, 5, 9, 13, and 17 weeks) samples stored in headspace vials at different relative humidities (0%, 11%, 33%, 54%, and 75%) were taken out from the storage box and were tightly closed with an appropriate silicon capsule. Two vials were added with 15 µL of hexanal and another two vials without solvent served as the blank. The headspace ampoules or vials were arranged in random order in the headspace GC auto-sampler (Perkin Elmer HS40XL). The GC (Perkin Elmer Autosystem XL) was programmed according to the standard parameters and conditions. Pressurization of the ampoules was carried out for 2 minutes, while thermostat time at 80 ºC for release of hexanal and propanal from the samples was 18 minutes. During this step, the injector needle fills the ampoule with helium. The pressure of the carrier gas (helium) was 12 psi. The injector and detector temperatures were 175 ºC and 250 ºC, respectively. The signal from the detector was passed to the ChemStation GC program

and recorded in the memory of the computer. Results were interpreted in the chromatogram with peak areas and retention time also stored in the computer system.

3.2.11 Morphology of spray dried powder by scanning electron microscopy

The cross-sectional structure of the powder particles was examined using field emission scanning electron microscope Model FEM-SEM (Leo DSM982 Gemini, LEO Electron Microscopy Inc., Germany). Operating voltage of 0.7 kV or 2.0 kV were used similar to the previous research by Partanen et al. (2008). The inside of the particle was ruptured by quick removal of adhesive tape fastened to the powder sample. Samples were covered in sputtered platinum to stabilize the particles and improve the contrast of the image when viewed under the electron beam (Partanen et al. 2008).

3.2.12 Analysis of carotenoid and chlorophyll

Flaxseed oil samples weighing 1g each were diluted in 10 mL heptane. Spectrophotometer was set to zero with heptane while choosing the suitable wavelength for each analysis. For carotenoid determination, the absorbance of oil solutions was measured at a wavelength of 450 nm using VIS disposable cuvettes. The concentration of carotenoid was computed using the Beer-Lambert law and taking into account the dilution factor.

The same dilution was used for flaxseed oil in the chlorophyll analysis while spectrophotometer was also set to zero with heptane. Absorbance of each sample was measured in three wavelengths (630 nm, 670 nm, and 710 nm). The amount of chlorophyll was calculated using the equation adapted from AOCS (1981).

3.2.13 Determination of total phenolic compounds by Folin-Ciocalteu method and UV spectroscopy

Following the same method used by Lampi (2009), extraction of flaxseed oil and sunflower oil was done by weighing 30g of the oil and washed with 30mL heptane. The solution was

transferred to a separatory funnel and added with the extraction reagent (80:20, methanol:water) until a clear separation between the lipid phase and aqueous phase is apparent. Parallel sample extracts were combined into round bottom flask and washed with another 30 mL heptane. The extract with solvent was then dried in a rotary evaporator at $\leq$ 35°C. Ethanol of about 10 mL was added intermittently to help evaporation of water. The dried residue was again dissolved in methanol and transferred to a 5mL volumetric flask and filled to the mark. The methanol extracts of 200 µL were pipetted into test tubes and evaporated under nitrogen gas and added with 200 µL Milli-Q water. A blank sample was prepared containing 200µL Milli-Q water for zeroing the spectrophotometer.

A standard curve was generated using gallic acid for quantification as shown in Table 5. A standard stock solution was made by dissolving 100 mg of gallic acid in 100 mL methanol. The solution was evaporated under nitrogen gas and filled the bottle to the mark with Milli-Q water.

Table 5. Standard series preparation for total phenols measurement

Gallic Acid, mg	Dilution	Gallic Acid stock solution, mL	Water, mL
5	1:200	0.025	5
10	1:100	0.050	5
20	1:50	0.10	5
30	1:33.3	0.10	3.33
40	1:25	0.10	2.50
100	1:10	0.50	5

Around 200 µL each of the standard solutions, diluted parallel extracts, and blank sample were pipetted into test tubes. For every standard solution, extracts, and blank sample 1mL of Folin's reagent, 0.8mL of 7.5%Na_2CO_3 were added. The tubes were mixed in vortex and allowed the solutions to stand for 30 minutes in the absence of light. Spectrophotometer was set to 765 nm and the blank sample was used to zero the equipment. After the samples have been stored for 30 minutes, absorbance of each samples was measured using VIS disposable cuvettes. Results were then calculated following the standard series in a Microsoft Excel.

3.3 Results

3.3.1 Chemical characterization of bulk and microencapsulated flaxseed oils

After the materials were stabilized at various relative humidities (0%, 11%, 33%, 54%, and 75%) for one week, they were characterized for their chemical properties (Table 6; Appendices 1-7). Individual results for chemical properties are also shown in Appendices 1-7. The initial peroxide value (PV) of flaxseed oil (FSO) at the start of the experiment was 0.25 meq/kg, and it increased a little to 0.4 – 0.5 meq/kg during the stabilization time.

The proportion of surface lipids (SL) was ca 5% of that of total lipids (TL). There was some variation in all chemical properties among the samples stabilized at different relative humidities. A negligible amount of palmitic acid in CL and NCL was observed at the initial stage of storage. Variations in PVs in SL and TL for both microencapsulated oils were noticeable after stabilization period.

Table 6. Chemical composition of flaxseed oil (FSO), microencapsulated FSO with non-cross-linked Na-caseinate (NCL) and microencapsulated FSO with cross-linked Na-caseinate (CL) after being stabilized at different relative humidities for one week.

Chemical properties	FSO *)	NCL	CL
Surface lipids (SL) in powder (mg/g)	993-1013	12.7-16.9	7.8-16.1
Total lipids (TL) in powder (mg/g)		240-295	272-293
Palmitic acid in SL (mg/g)	48.4-49.3	0.6-0.8	0.4-0.8
Palmitic acid in TL (mg/g)		12.5-14.4	12.4-13.5
α-linolenic acid in SL (mg/g)	591-603	7.5-10.0	4.6-9.6
α-linolenic acid in TL (mg/g)		149-177	160-178
γ-tocopherol in SL (µg/g)	452-476	356-425	418-446
γ-tocopherol in TL (µg/g)		414-435	450-489
Peroxide value in SL (meq/kg)	0.4-0.5	0.2-8.2	4.3-6.7
Peroxide value in TL (meq/kg)		0.7-4.7	1.8-5.9

*) results of FSO were measured from heptane-dissolved FSO and reported as concentration in TL

In addition, flaxseed oil was examined for the presence of phenolic compounds. An average of 288 µg/100 g was found in the commercial FSO. Likewise, there was considerable amount of

carotenoid content (14 µg Carotenoid/ g oil) in FSO. The chlorophyll content for FSO was insignificantly lower with 0.3 µg Chlorophyll/ g oil.

.

3.3.2 Powder morphology of microencapsulated flaxseed oil materials and their changes during storage

Using field-emission scanning electron microscopy, morphology of powder particles was examined. Figures 5-10 and Appendix 8 showed the microstructures of encapsulated oils indicating large and small oil droplets. Figures 5 and 6 showed cross-sectional view of the spray dried oils stored for 2 weeks (both CL and NCL at 11%RH and 54%RH) which were solid, porous, and powder particles were mostly globular with few creases on the surface. The powder particles prepared by high pressure homogenization of emulsion clearly entrapped larger oil droplets. After 2 weeks storage both CL and NCL showed collapse, sticky or rubbery structure at high moisture content (75%RH) (Figure 7). Prolonged storage of powdered samples after 10 weeks for CL and 17 weeks for NCL revealed slight difference at 11%RH (Figure 8), more creases developed at the surface of powdered particles. Large holes were found for CL and NCL stored after 10 and 17 weeks respectively at 54% RH (Figure 9) and 75%RH (Figure 10).

For the visual appearance of CL and NCL powder particles, Figure 11 showed enlarged photos of powder particles after the end of stabilization period. There were noticeable differences among powder materials stored at 75%RH, powder particles tend to transform into sticky agglomerates for CL and NCL due to accumulation of moisture and color was more yellowish compared to other samples. For the CL, samples changed into aggregates but not sticky or gummy in texture at 54% RH. The CL samples remained in porous form at 0%, 11%, and 33%RHs. Slight difference for NCL was evident in powder particles, wherein materials remained porous at 0%, 11%, 33%, and 54%RHs (Figure 11).

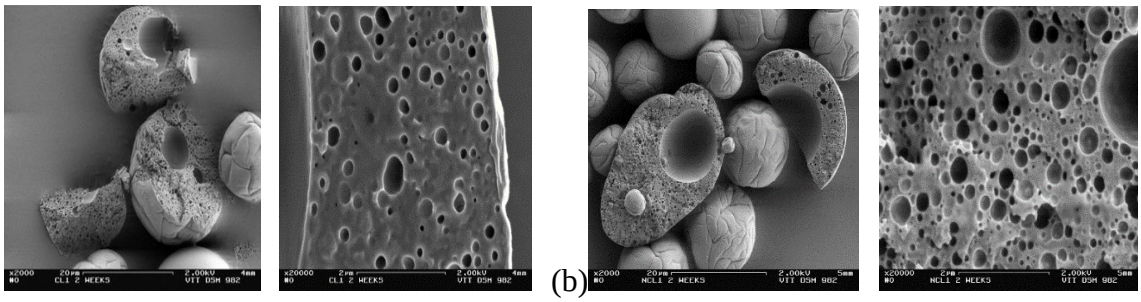
(a)  (b)

Figure 5. SEM of CL (a) and NCL (b) flaxseed oil powder stored 2 weeks @RH 11%.

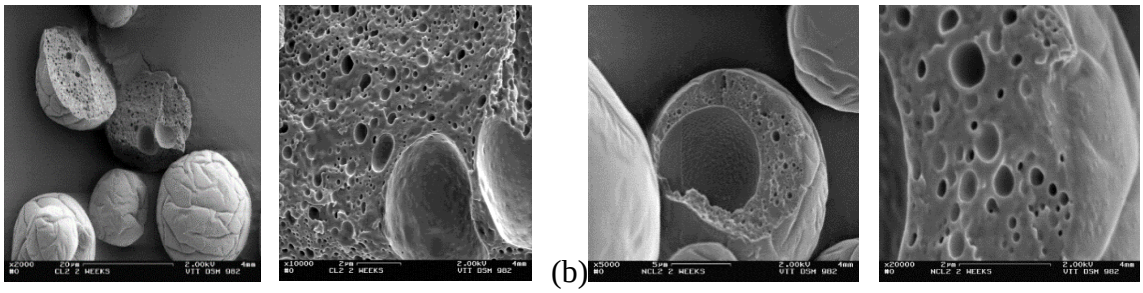
(a)  (b)

Figure 6. SEM of CL (a) and NCL (b) flaxseed oil powder stored 2 weeks @RH 54%.

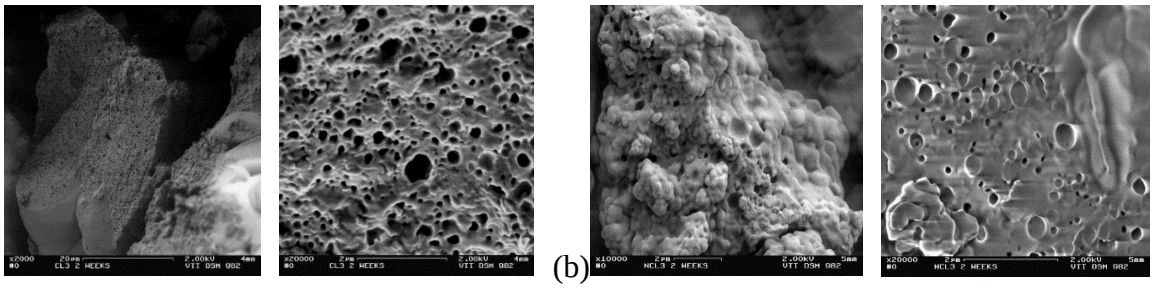
(a)  (b)

Figure 7. SEM of CL (a) and NCL (b) flaxseed oil powder stored 2 weeks @RH 75%.

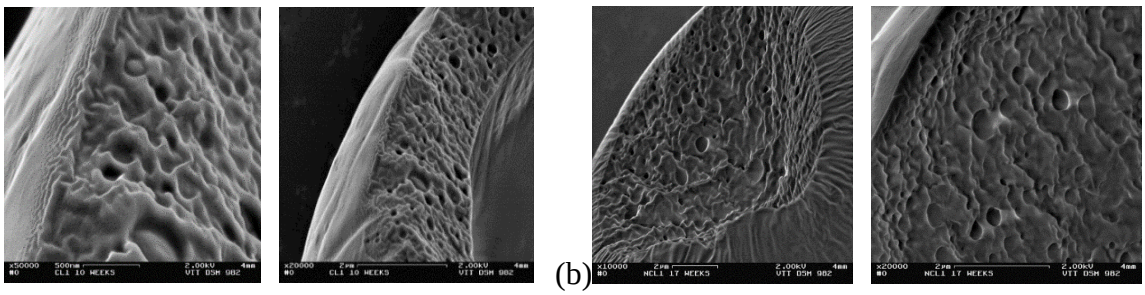
(a)  (b)

Figure 8. SEM of CL 10 weeks (a) and NCL 17 weeks (b) flaxseed oil powder stored @RH 11%.

(a) (b)

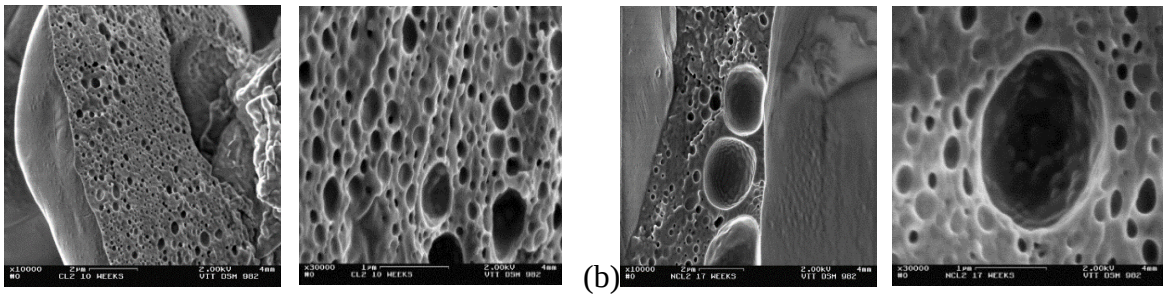


Figure 9. SEM of CL 10 weeks (a) and NCL 17 weeks (b) flaxseed oil powder stored @RH 54%.

(a) (b)

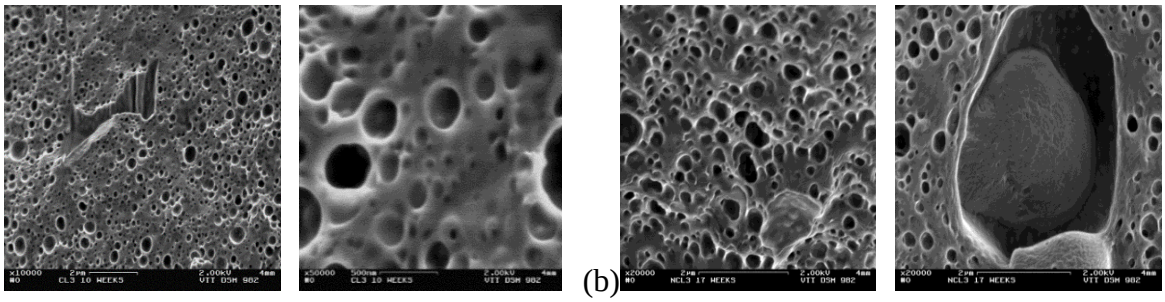
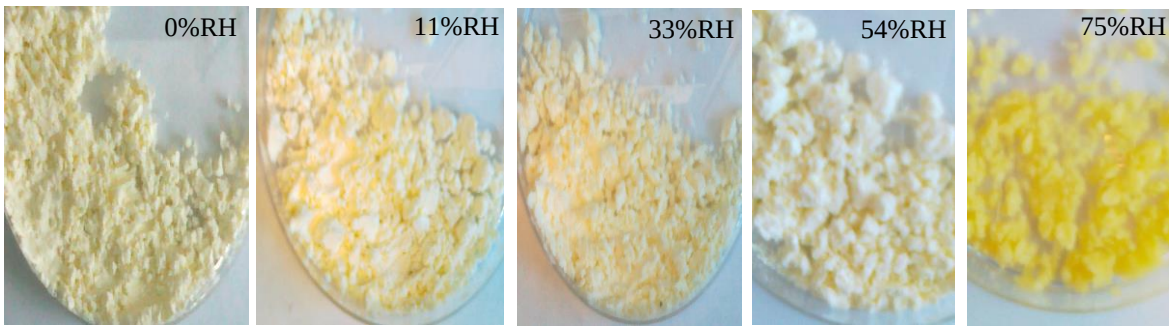


Figure 10. SEM of CL 10 weeks (a) and NCL 17 weeks (b) flaxseed oil powder stored @RH 75%.

(a)



(b)

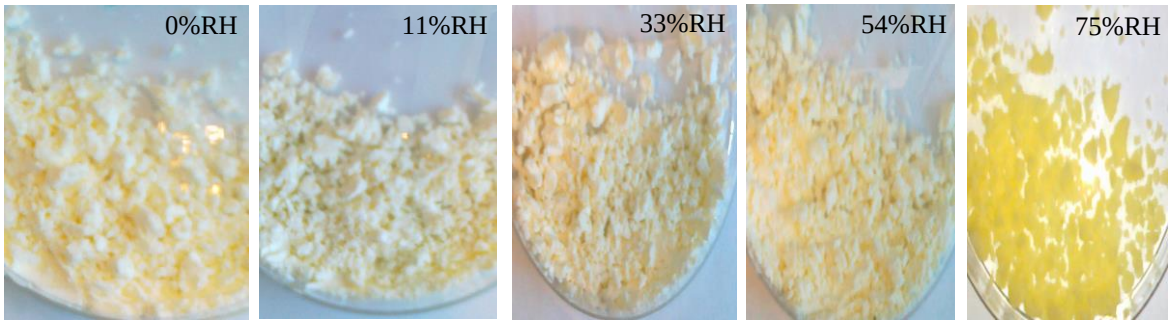


Figure 11. Cross-linked (a) and non-cross linked (b) microencapsulated flaxseed oils after 17 weeks stabilization.

3.3.3 Changes in chemical properties of bulk and microencapsulated flaxseed oil during storage at different relative humidities

The oxidative stability of microencapsulated flaxseed oil by NCL and CL Na-caseinate with maltodextrin as the matrix was further investigated. The chemical properties of dried emulsion as a function of lipid oxidation were measured for each time point.

The bulk oil was very stable during the storage (Figure 12). The PVs (0.4-1.7 meq/kg) were extremely low throughout the whole storage period in comparison to the oils in both microencapsulated materials. The bulk FSO was more stable than CL and NCL, and there was no evidence of oxidation even after storage period (Figure 12).

Formation of hydroperoxides as a primary product of oxidation and an indication of oxidative deterioration in lipids was studied. The acceptable PV in fats and oils is 10 meq/kg and above this range lipid oxidation and rancidity starts to progress as reported in Codex Alimentarius Standards. The PVs of surface oil in CL showed a constant increased rate of oxidation at 0% RH for 17 weeks storage (Figure 12). The same oxidative instability was observed in NCL at 0%RH. Surface oils in CL and NCL started to oxidize after 3-weeks storage, PVs exceeded the acceptable standard for oils of 10 meq/kg. The highest peroxide values for total lipids in both CL and NCL were 70.7 meq/kg and 70.9 meq/kg at 33% RH at the end of storage. CL dried emulsion was less stable at 0% RH compared to NCL, as evident in their peroxides in CL (159.7 meq/kg) and NCL (71.5 meq/kg) after storage.

At highest moisture conditions (75%RH), CL was stable and did not oxidized even after stabilization period. TL in the NCL samples were stable at 75%RH, but in SL only until 13 weeks (Figure 12). SL in both CL and NCL were oxidative stable until 9 weeks storage at 54% RH, while TL in both microencapsulated oils showed good stability throughout storage period at 54% and 75% RHs.

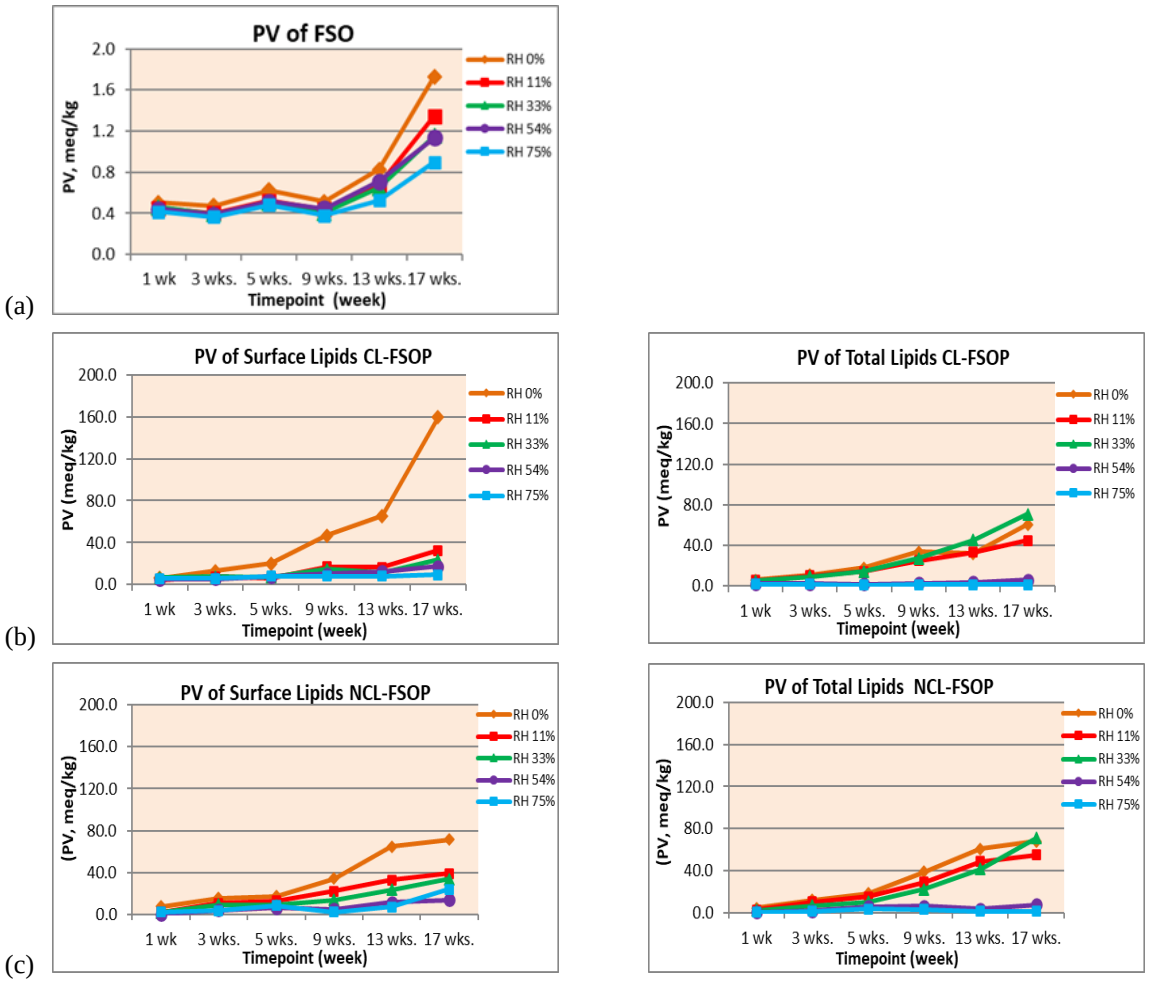
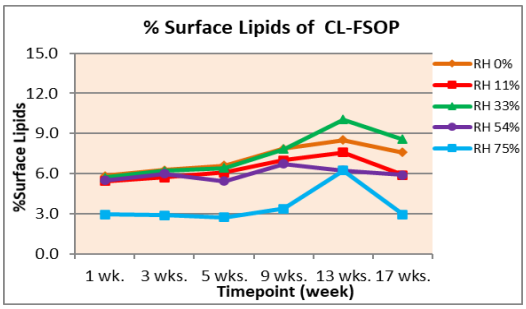


Figure 12. Peroxide values (meq/kg) of bulk oil (a), surface and total lipids in cross-linked (b) and non-cross-linked (c) microencapsulated flaxseed oil stored at different relative humidities

Figure 13 showed changes of SL contents through storage time. In general, there were no major changes in the proportion of the SL during storage and the lowest values were found in samples stored at 75% RH. There was a lower proportion of SL in CL at 75%RH compared to NCL at the same humidity condition. By the end of storage point, CL had the highest surface lipids (8.6%) at 33%RH while in NCL it was 8.9% at 0%RH. There was only slight change in the SL for both microencapsulated powders after 17 weeks storage. Efficiency of microencapsulation is based on the portion free oil or unencapsulated oil after processing of dried microencapsulated oil.

(a)



(b)

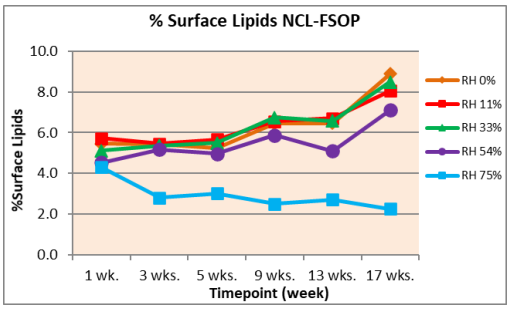


Figure 13. Surface lipids content (%) cross-linked (a) and non-cross-linked (b) microencapsulated flaxseed oil stored at different relative humidities

To follow the progress of lipid oxidation among CL, NCL, and FSO, measurement of secondary oxidation products (hexanal and propanal) was performed by static headspace gas chromatography. For FSO, there was no hexanal content in other RHs (0%, 33%, 54%, and 75% RHs) while at 11%RH a small amount of hexanal was observed after 5-weeks (219.9 uV*s) and 9-weeks (403.8 uV*s) of storage. Flaxseed oil was different from the two microencapsulated oils, varying results were evident in which high propanal content was noticed after 3-weeks at 33%RH (2734.1 uV*s) and at 75%RH (3189.8 uV*s) and subsequently decreased until the storage was completed (Figure 14).

Hexanal concentration was highest in dry condition (0%RH) for CL (596.3 uV*s) and low moisture (11%RH) for NCL (1227.7 uv*s), although the amount was not too high compared to propanal. Negligible amount of headspace hexanal was detected in both CL and NCL powders at 75%RH. Both microencapsulated oils produced high propanal contents at intermediate moisture 33%RH with 32,597.4 uV*s for NCL while 25,264.4 uV*s for CL. At high moisture (54% and 75%RHs) and low moisture (0% and 11%RHs) conditions, only slight propanal formation was detected at the end of storage (Figure 14).

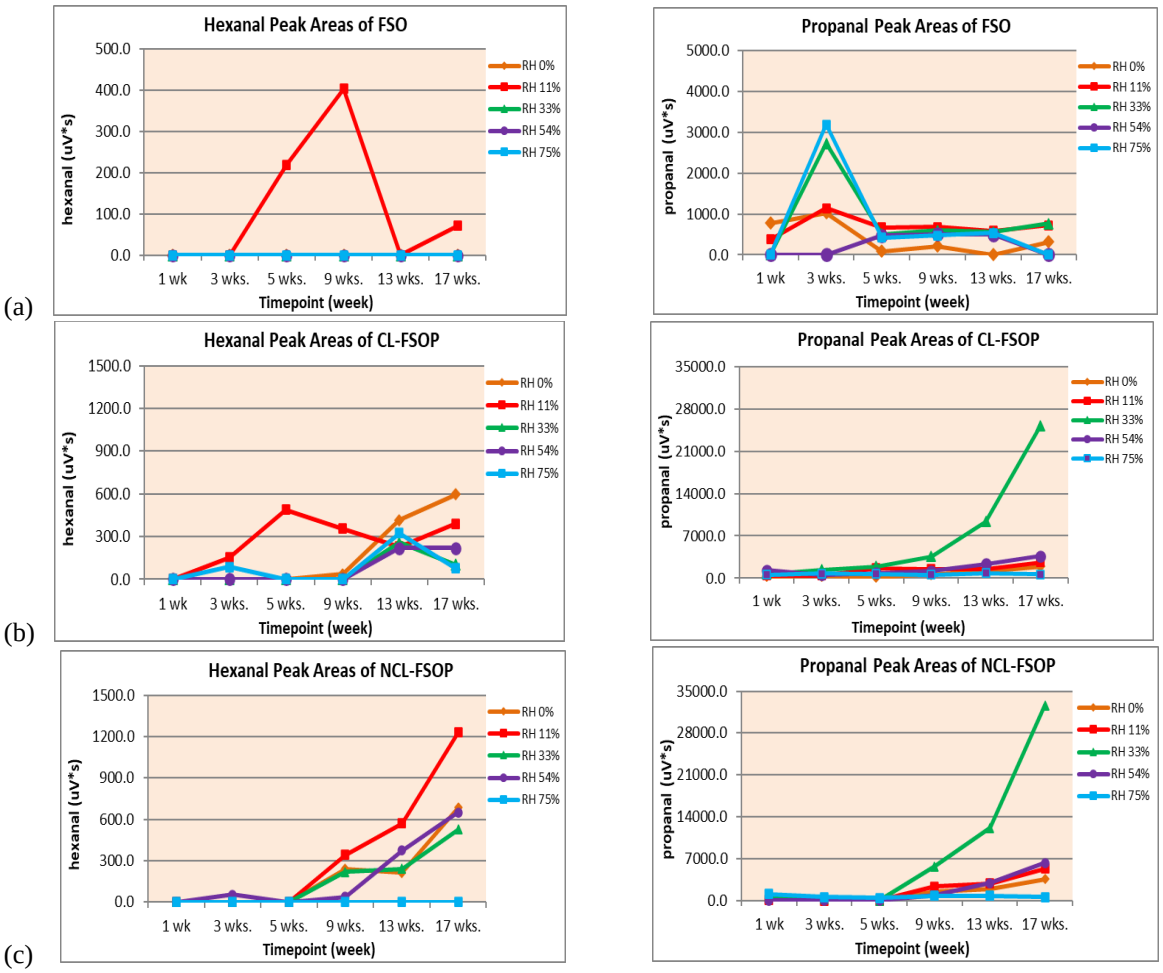


Figure 14. Hexanal and propanal (uV*s) of bulk oil (a), cross-linked (b) and non-cross-linked (c) microencapsulated flaxseed oil stored at different relative humidities

The fatty acid (FA) content of flaxseed oil was high (992.8-1004 mg/g) after 1-week storage and only a small decrease in FA content was noted throughout the storage period and in all RHs (0%, 11%, 33%, 54% and 75%) (Figure 15). This explains that the commercial FSO utilized was relatively stable and could be relatively due to the smaller surface area of the oil compared with dried emulsions.

Overall, the FA and ALA content (Figure 15 and 16) of CL and NCL microencapsulated FSO behave similarly in surface and total fraction of the oils wherein samples had only slight differences in the amount throughout the storage time. It was only at 75%RH in surface oils where FA slightly reduced by 37.2% for CL and 54.3% for NCL from 1-week until the end of storage.

On the other hand, the lowest fatty acid was found at 75%RH for surface fraction in CL and NCL after 17 weeks. The highest (75%RH) and intermediate (33%RH) moisture conditions catalyzed the reduction of FA to a lesser extent in both microencapsulated oils (Figure 15).

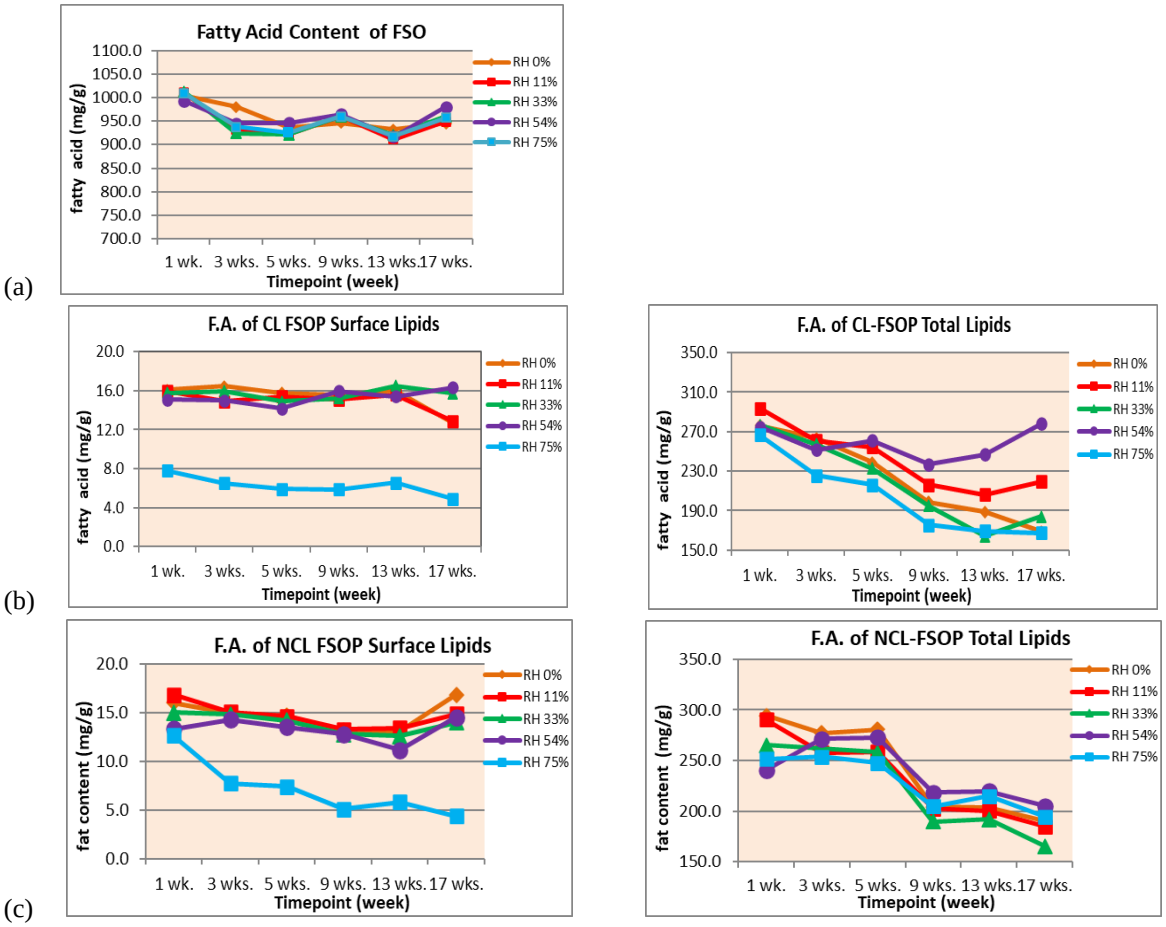


Figure 15. Fatty acid content (mg/g) of bulk oil (a), cross-linked (b) and non-cross-linked (c) microencapsulated flaxseed oil stored at different relative humidities.

A significant concentration (543-603 mg/g) of ALA in FSO was considerably stable at different RHs and storage periods. The amount lowered after 5- and 13-weeks storage but then increased at the final stage of storage. There was only 5.8% reduction of ALA in FSO at 0%RH where the lowest degradation occurred. The ALA of bulk oil did not vary a lot which also proved its stability over RH conditions (Figure 16).

In general, high amount of ALA (ca 177 mg/g) and only small portion decreased during storage at various humidity conditions in both microencapsulated oils. Increasing the relative humidity up to 75%RH also showed loss of ALA in the surface fraction of CL and NCL. For CL (0%,

33% and 75% RHs) and NCL (0%, 11% 33%, 54% and 75% RHs), there was a gradual decrease in ALA over 17-weeks storage in TL. In terms of total oil, the lowest ALA was at 0%RH for CL while 33%RH for NCL (Figure 16).

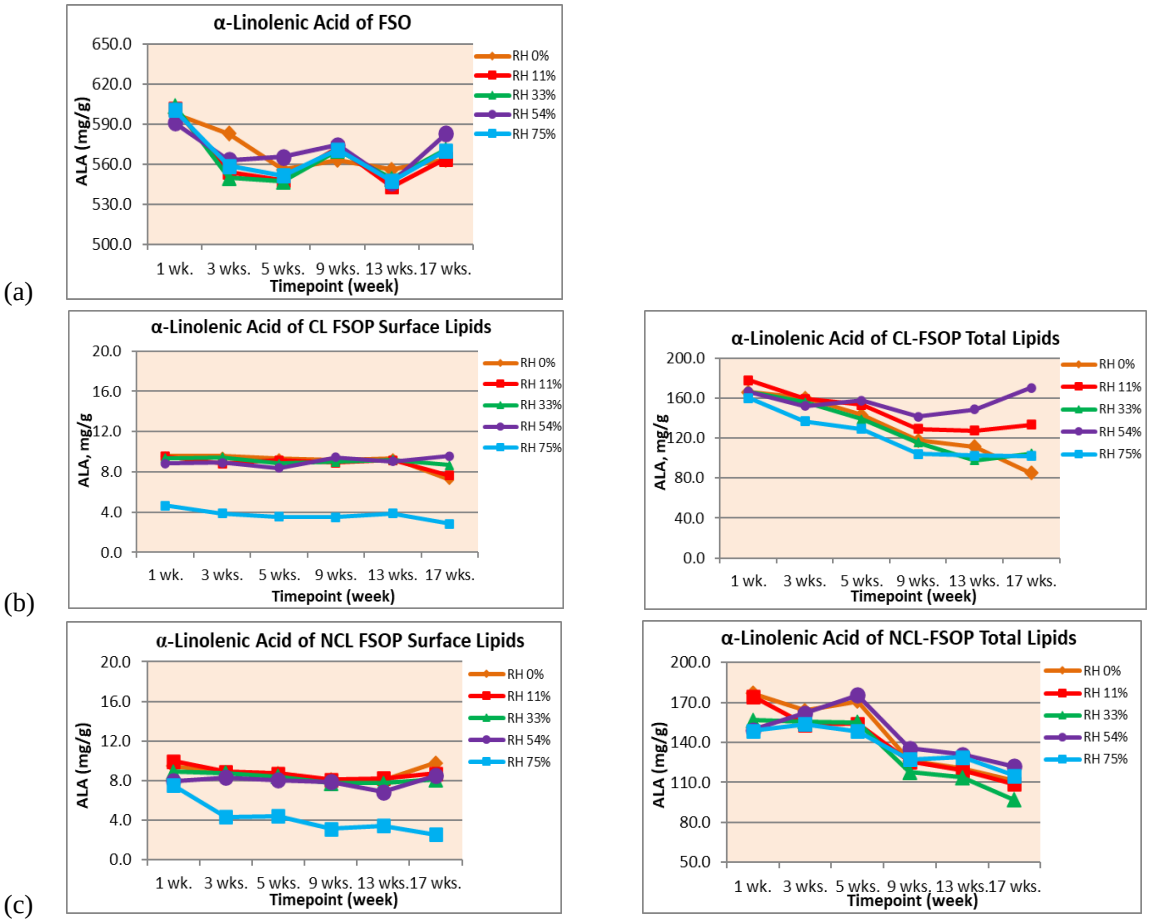


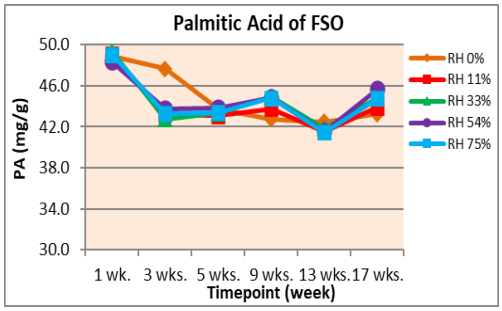
Figure 16. α-linolenic acid (mg/g) of bulk oil (a), cross-linked (b) and non-cross-linked (c) microencapsulated flaxseed oil stored at different relative humidities.

In the case of FSO, there was only small concentration of palmitic acid detected between 41.4-49.1 mg/g. The same result of palmitic acid content was observed in all RHs which lowered after 3 weeks then increased again after 17 weeks storage. Palmitic acid was mostly stable in different RHs except at 0%RH where a small decrease from 48.9 to 43.2 mg/g was noticed (Figure 17).

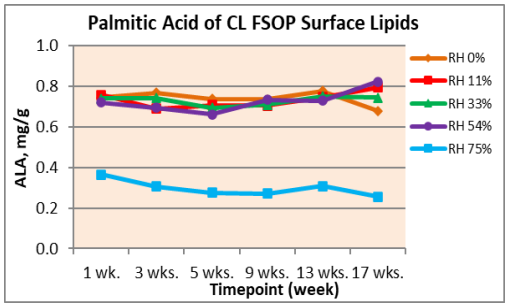
Generally, palmitic acid was stable and only a slight decrease at moist conditions for CL and NCL for surface and total lipids. A similar decreasing pattern of palmitic acid loss at 75%RH was noted in CL and NCL for surface fractions (Figure 17). For TL reduction in content after

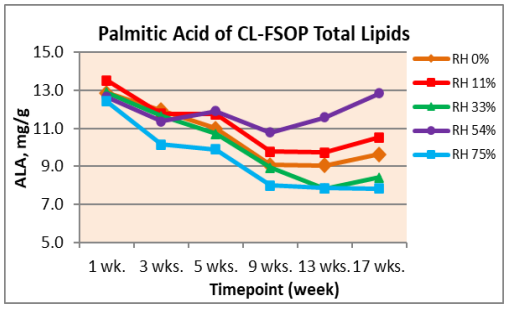
17 weeks was lowest at 75%RH for CL while 33%RH for NCL. Only slight losses of 37.1% for CL and 40.2% for NCL in total lipids at the end of storage. Although the amount in CL for total oils was fluctuating after 5, 9, 13, and 17 weeks storage (Figure 17).

(a)

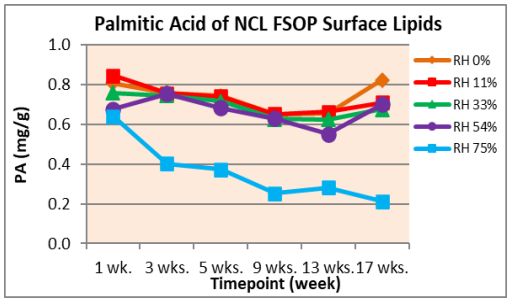


(b)





(c)



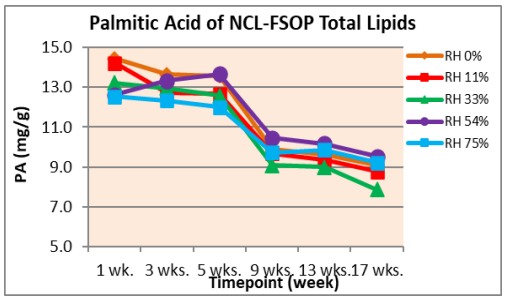


Figure 17. Palmitic acid (mg/g) of bulk oil (a), cross-linked (b) and non-cross-linked (c) microencapsulated flaxseed oil stored at different relative humidities.

Gamma (γ)-tocopherol is the major antioxidant contained in flaxseed oil. The γ-tocopherol content of the FSO remained stable throughout the storage, and the relative humidity did not have great influence on the content. There was only 5.8% reduction based on the initial concentration of γ-tocopherol for FSO after 17 weeks storage. Results were fluctuating at 75%RH, the amount decreased a little after 9 weeks but increased again by the end of stabilization (Figure 18).

Overall, degradation of γ-tocopherol in CL and NCL particularly in the surface oils was insignificant although minor loss was evident at dry conditions (Figure 18). The loss of γ-tocopherol correlates to previously reported peroxidation in surface oils for CL and NCL wherein highest PVs were demonstrated in samples stored at 0%RH. For CL, there was 96.7% of γ-tocopherol loss which was comparatively higher than 83.2% loss for NCL in SL at the end of the storage. Only small reduction in γ-tocopherol was observed at moist conditions (33%, 54%, and 75%) for CL and NCL (Figure 18).

For TL, a slight difference of γ-tocopherol in CL and a 12.7% increased occurred at 54%RH after storage. In the NCL, a small decrease of 31% in tocopherol at 0%RH after 17 weeks was observed. At the highest humidity (75%RH), both microencapsulated oils were generally stable during the entire storage (Figure 18).

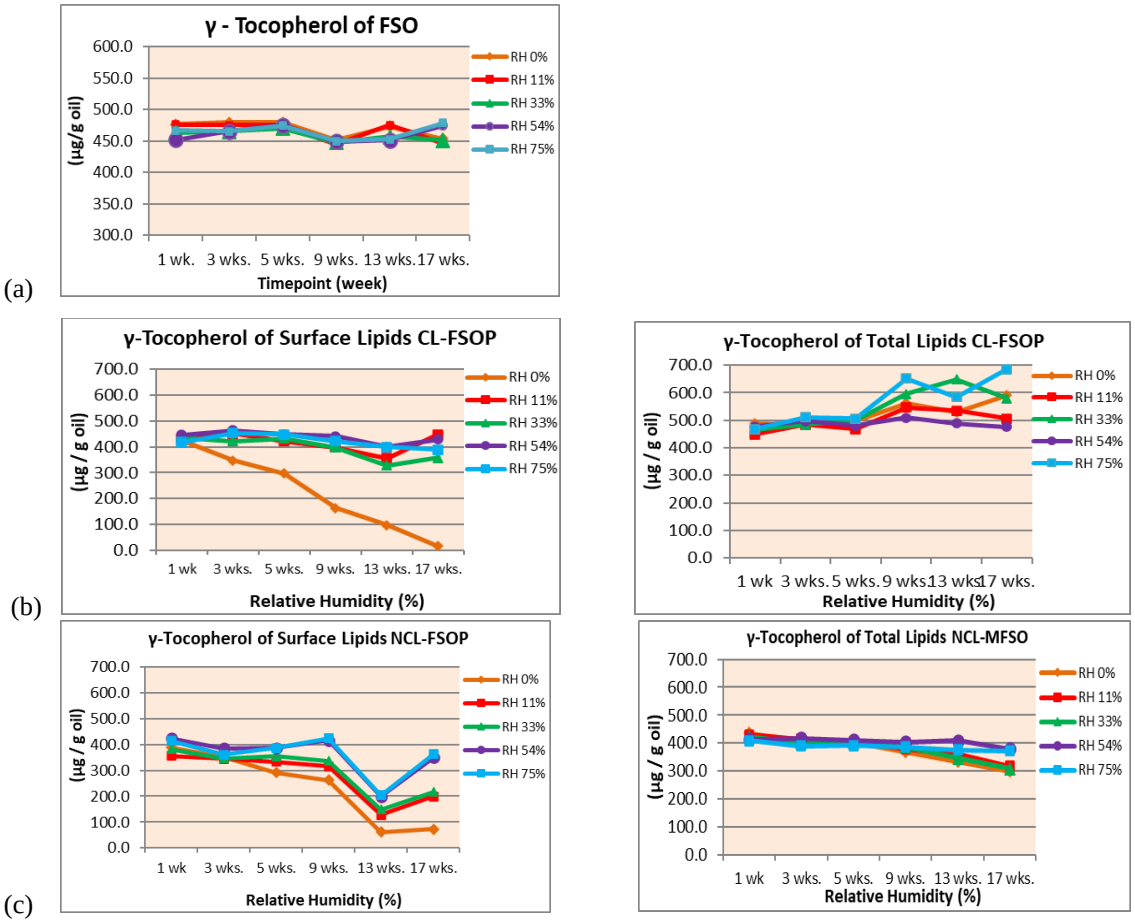


Figure 18. γ-Tocopherol (µg/g oil) of bulk oil (a), cross-linked (b) and non-cross-linked (c) microencapsulated flaxseed oil stored at different relative humidities.

3.4 Discussion

3.4.1 Evaluation of microencapsulation efficiency

As previously presented in the results, oxidation rate increased as the relative humidity decreased. At dry storage conditions (0%RH) PV was highest for both CL and NCL dried emulsions. The PVs were twice as much higher in CL than in NCL after 17 weeks storage. This could be attributed to low encapsulation efficiency for microencapsulated oils, as more surface lipids were formed and oxidized in CL. As reported earlier, the initial surface lipids for CL (5.03%) was slightly higher than NCL (4.84%). The encapsulated oil surrounded by the protein interface and carbohydrate matrix makes up for the total lipids which were 94.97% for CL and 95.16 for NCL. The free oil is more sensitive to oxidation than the oil globules protected by the matrix and interface (Velasco et al. 2003; Velasco et al. 2009).

At the end of storage time, surface lipids had higher PV than total lipids at the same RH (0%). The SL also known as free oil or unencapsulated oil is much more sensitive to lipid oxidation when exposed to oxygen compared to the encapsulated oil protected by the matrix (Velasco et. 2003; Tonon et al. 2011). At high moisture content 54% and 75% RH, oxidation rate was lowest which was similar to the study by Partanen et al. 2008 with flaxseed oil and by (Damerau et al. 2014) with sunflower oil. This can be explained with the change in structure of powder particles. In studies by Moisio et al. 2014 and Damerau et al. 2014, at 75%RH structure of dried emulsion transformed from amorphous or glassy state into collapsed or gummy structure. High water saturation allowed structure transformation of dried emulsion into aggregates and formation of cavities for oxygen transport. At high relative humidities, particles tend to lose its gas phase cavities and become compact. Therefore, there is decreased oxygen availability for powder particles because fast oxygen diffusion changes into slower form (Partanen et al. 2008; Damerau et al. 2014; Moisio et al. 2014;).

In studies by Moisio et al. 2014 and Damerau et al. 2014, encapsulation efficiency was directly influenced by the protein content at the surface such that optimization of the protein is important to achieve the smallest amount of surface lipids. According to Tonon et al. (2012) higher surface or unencapsulated oil is the result of greater oil concentration due to lower encapsulation efficiency. Minimoto and others (2002) also revealed as the weight ratio of core in wall material increased, encapsulation efficiency decreased in the encapsulation of linoleic acid. The low

encapsulation efficiency is due to inadequate amount of wall material to completely protect the oil particle.

Overall, the surface oil concentration resulted to higher PVs at dry storage condition. Low encapsulation efficiency led to higher amounts of surface oil, which was more susceptible against lipid oxidation when in contact with oxygen. A small proportion of surface lipid was essential to powdered oil stability because oil globules on the particle surface are less protected against oxygen (Minimoto et al. 2002; Partanen et al. 2002; Tonon et al. 2012).

3.4.2 Evaluation of relative humidity on the stability of microencapsulated oil

In this study humidity conditions caused changes on the chemical and physical stability of encapsulation interface and matrix. At dry conditions (0%RH) spray dried emulsion remained in its porous or powdered form thus promoted rapid atmospheric oxygen diffusion in between the gas phase of powdered emulsion. This made oxygen readily available at the powdered oil surface, hence more prone to oxidation. The formation of creases, pores, or wrinkles at the surface of powder particles allows oxygen diffusion and transfer to oil globules (Hardas et al. 2002; Damerau et al. 2014). The larger surface area of powder particles and cracking of matrix in CL and NCL also allow oxygen diffusion at low moisture and dry conditions; thus, oxidation rate was high. The maximum rate of oxidation at 0% RH was also attributed to solvation and stabilization of the propagation state by water. Increasing the relative humidity beyond 0% resulted in solvation of the peroxy radical, thus, hindering the radical from entering the propagation state (Kahl et al. 1988; Frankel 1998; Belitz et al. 2004; McClements and Decker 2008; Coultate 2009).Sensitivity of PUFAs in flaxseed oil is due to its chemical configuration because they contain many bis-allylic double bonds which are prone to lipid oxidation (Mc Clements and Decker 2008).

Hydroperoxide is a primary product of lipid oxidation and this was used as an indicator of oxidative deterioration in this study. As reported in Codex Alimentarius Standards, the acceptable PVs for fats and oils is 10 meq/kg and above this range product becomes rancid and unacceptable. The formation of hydroperoxides from PUFA may cause double bond isomerization. Aside from PV analysis, the determination of conjugated dienoic and trienoic

fatty acid derivatives also allow assessment of oxidation in food products (Waraho et al. 2011; Vieira et al. 2017).

At high moisture storage, PVs were lowest and the chemical properties of the matrix of microencapsulated oils tend to be more stable, but the physical structure may be unacceptable to consumers. According to the studies reported by Moisio et al. 2014 and Damerau et al. 2014, at 75%RH structural transformation took place in dried emulsions where powdered particles became collapsed and altered into gummy structure. High water content permitted structural transformation of dried emulsions (CL Na-caseinate and NCL Na-caseinate with maltodextrin as the matrix) into aggregates and allowed cavity formation for oxygen transport. Structure of dried emulsions transformed from amorphous or glassy state into sticky form at high moisture conditions. High water saturation allowed structural transformation of dried emulsion into aggregates and formation of cavities for oxygen transport. At higher relative humidities, powder particles may lose its gas phase cavities and become compact as evident in the visual appearance shown earlier. As a result, there was decreased oxygen availability for powder particles due to rapid oxygen diffusion that changes into slower form (Partanen et al. 2008; Damerau et al. 2014; Moisio et al. 2014).

According to McClements and Decker (2008), the number of peroxides present in vegetable oil is an index of its primary oxidative level and consequently its tendency to go rancid. The PVs of the samples also determine that temperatures, exposure to oxygen and storage time are the chief factors that contributed to the oxidation of the dried microencapsulated samples. In the present study, PVs was influenced by the moisture induced condition. In this case, unsaturated free fatty acids reacted with oxygen and formed peroxides which determined a series of chain reactions that generate the formation of volatile substances accelerated by high temperature, relative humidity, and oxygen exposure. The principles of the spectrophotometric measurement of peroxide value explains that the ROOH peroxides oxidize and react with Fe (II) ions and the Fe (III) ions following oxidation are grouped and form a red complex. This was also proven in the test when a distinct red violet color was observed from the sample mixture during addition of the Fe (II) Cl_2 (Frankel 1998; Hornero-Mendez et al. 2001; Belitz et al. 2004; McClements and Decker 2008; Coultate 2009).

On the other hand, linoleic acid and ALA is the precursor of peroxidation byproducts, in this study hexanal and propanal were used as an indicator for the progress of secondary products of

oxidation in microencapsulated oils. Secondary oxidation products are formed when fatty acid hydroperoxide decompose through β-scission reactions. The reactions can produce various volatile and nonvolatile compounds from lipid oxidation. In some cases, the presence of antioxidants can influence the amount of secondary products which can be minimal while primary oxidation product can be high (Mc Clements and Decker 2008) Amount of hexanal values for this study showed the release of hexanal was twice as much in NCL dried emulsion than in CL mixtures. According to reports, the physical changes of the solid matrix of microencapsulated oils may affect the oil distribution in which the partial release of encapsulated oil and released oil may be more exposed and undergo rapid oxidation. The relative humidity or water activity in the food matrix was used to investigate on the rate of release of hexanal and propanal at different RH conditions. This explains the relationship between lipid oxidation and moisture content. In general as shown in the low release of headspace hexanal and propanal, the CL samples exhibited a protective effect of the capsule against extreme oxidation due to the changes made in the physical structure of the food matrix (Kahl et al. 1988; Frankel 1998; Lewandowicz et al. 2005; McClements and Decker 2008).

Hexanal released was more predominant at 0% RH for CL and 11%RH for NCL, while for propanal both CL and NCL had the highest peak areas at 33%RH. At 0%, 11%, and 33% RH, the structure of the sample is dry due to the presence of low moisture and the release of hexanal is not so high. At high humidity condition (75% RH), the structure seems to be collapsed as evident in the physical appearance, the samples were sticky and hard which enables the hexanal to stay in the flaxseed oil. This also explains that hexanal is less soluble in water, based on its solubility value of 6g/L at 20^0C (Lewandowicz et al. 2005; Frankel 1998; McClements and Decker 2008). In this experiment, the formation and release of hexanal and propanal compounds were measured because the sample vials were open during the entire storage and some of the volatile compounds may have been released earlier.

The Na-caseinate interface layer of both microencapsulated flaxseed oils in this study proved to be influenced by moisture induced changes as evident in the variations of chemical and physical properties. Proteins have surface active characteristics which acts as emulsifiers and forms an interfacial layer on surface of oil globules. Proteins can easily react with lipids and carbohydrates, and they are known to reduce lipid oxidation by quenching radicals. By reacting with both primary and secondary products of oxidation, proteins in dried emulsion may impact

chemical properties and structural transformation through polymerization, cross linking, or scission reactions (Schaich and Karel 1976; Schaich 2008; Damerau et al. 2014). As reported by Vega and Roos (2006), storage stability of dried dairy emulsion is related to lactose crystallization and release of encapsulated materials stored at high moisture condition 75%RH. Accordingly, the chemical composition of the encapsulated emulsion influenced the properties of the bulk stability, wettability, and flowability.

Generally, the high amount of ALA and γ-tocopherol in CL and NCL microencapsulated oils showed only minor degradation in contents throughout storage at different moisture conditions. The loss of γ-tocopherol relates to previously described PVs in surface oils for CL and NCL in which highest PVs stored at 0%RH were noticeable. However, γ-tocopherol in other humidity conditions (11%, 33%, 54%, and 75%RHs) were stable all throughout the stabilization period. As reported in literature, tocols offer an antioxidant activity in FSO by protecting the MUFAS and PUFAs from oxidation. This also explains the high amount of these phenolic compounds in highly unsaturated edible FSO. Tocopherols obstruct the hydroperoxide intermediates and prevent the autoxidation chain reaction (Tuberoso et al. 2007; Hasiewicz-Derkacz et al. 2015; Shahidi and de Camargo 2016)

3.4.3 Morphology of dried emulsions

It showed that structural and morphological changes were noticeably clear in both samples under field-emission scanning electron microscope. When stored at moist conditions powder particles tend to transform into agglomerates, this is due to rearrangement of cavities between powder particles thus allowing less oxygen transport or diffusion on the surface. This led to stability against oxidation at 75%RH (Partanen et al. 2008; Damerau et al. 2014).

According to Mc Clements and Decker 2008, when water is removed from the food system lipid oxidation is lowered because of reduced mobility of reactants like transition metals and oxygen. Continuous removal of water accelerates lipid oxidation at water activity equal to or less than 0.3. This is because of loss of protective water solvation layer surrounding lipid hydroperoxides. An increase in surface area of particles also promotes exposure to prooxidants and oxygen thus, oxidation rate is also increased.

During prolonged storage at 0%, 11%, 33%, and 54% RHs, the results suggest that physical structure of dried microencapsulated oils remained unchanged. Particles stayed in its powdered or porous state and no agglomeration was detected in dried emulsions. Labuza (1980) studied the effects of water activity on reaction kinetics in food, they reported that the presence of moisture inhibits lipid oxidation by hydrogen bonding a portion hydroperoxide formed in the propagation step. During further reaction hydroperoxides are eliminated, a faster increase in oxidation rate takes place. This inhibitory effect of water on lipid oxidation is due to hydration of trace metals and decrease of its catalytic effect resulting to reduced effective metal concentration (Labuza et al. 1971; Labuza 1972; Labuza 1980; Velasco et al. 2009). It has been known that lipid oxidation in dried emulsions is influenced by humidity conditions. At extreme dry and humid conditions lipid oxidation develops quickly. At intermediate moisture concentrations equivalent to the monomolecular water layer, lipid oxidation progresses to a lowest rate （Labuza 1980; Velasco et al. 2009).

Various studies have reported the efficacy of microencapsulation of oils within a polymeric wall material while protecting the shelf life of PUFAs from the adverse effects of lipid oxidation (Frankel 1998; Partanen et al. 2005; Drusch et al. 2009; Tonon et al. 2011). In this study, results proved that microencapsulation of flaxseed oil and protein crosslinking contributed to storage and oxidative stability of dried emulsion at moist conditions. The same result was reported by Partanen et al (2008) and Klinkesorn et al. (2005) that encapsulation had considerably improved the storage stability of oxidizing oils like flaxseed oil and fish oil.

Protein cross linking and modification are frequently utilized to establish the functions of each amino acid side chains in the physical, chemical, and biological properties of proteins. In the production of functional food ingredients, it is therefore important to examine the harmful effect of exposure to interfaces on the structural and oxidative stability of proteins in emulsions (Buchert et al. 2010; Tonon et al. 2011).

As suggested by other authors, some oil droplets may oxidize rapidly once the oil is encapsulated in a glassy food matrix, while other oil particles may oxidize more slowly due to the heterogeneity in the encapsulation. In the glassy state there is less free space in comparison to the rubbery state as indicated to slow down diffusion of oil particles and lipid oxidation (Andersen et al. 2000; Partanen et al. 2005; Tonon et al. 2012)

3.4.4 Evaluation of stability of flaxseed oil

In the present study, the FSO was unexpectedly stable and it was difficult to figure out the reasons of its stability under different relative humidity conditions and storage times. The results were unpredictable and fluctuating in some properties like hexanal, propanal, and ALA contents. The major drawback of the research was difficulty in predicting the stability of FSO and compare it with microencapsulated oils due to the presence of antioxidative effects of phenolic compounds, carotenoids, and γ-tocopherol.

In plant oils like FSO, tocopherol is not the only redox active substances because other nonpolar polyphenolic compounds that can be extracted into oil during processing from plant seeds may also provide to antioxidant potential to oils (Prevc et al. 2015). This considerable amount of tocopherol can be accounted for the stability of the FSO against lipid oxidation. As previously stated, the bulk FSO was stable because there was negligible reduction of ALA and γ-tocopherol in all RHs. Low PVs, hexanal, and propanal in FSO also suggested low oxidation rate and this also explains the results why secondary oxidation products were exceptionally low compared to the microencapsulated oils. According to Herchi et al. 2011 the lipids present in flaxseed is protected against oxidation because of the presence of antioxidants like lignans and phenols. The small surface area of the oil limits oxygen diffusion which also explains the stability of FSO under varying humidity conditions and timepoints.

The presence of phenolic compounds in bulk FSO explained the stability of commercial oil against oxidation. The presence of antioxidants such as lignans and phenols in flaxseed allows protection against oxidation. Flaxseed oil contains high amount of ALA, tocopherol and has low glucosinolate content. High tocopherol content in FSO offer efficient protection against oxidation of unsaturated fatty acids in unstable oils (Herchi et al. 2011).

The high carotenoid content in flaxseed oil demonstrated oxidative stability. The presence of large number of conjugated double bond in carotenoids provides antioxidant protective effect by obstructing the hydroperoxide intermediates and inhibiting the oxidation mechanisms. Carotenoids can also quench singlet oxygen and inhibit cellular oxidative damage. The antioxidant properties of carotenoids have beneficials effect to health by lowering the risk of cancer, cataracts, atherosclerosis, and aging (Schwartz et al. 2008; Herchi et al. 2011).

As reported in this study, the chlorophyll contents of flaxseed and sunflower oils were negligible. Li et al. (2019) studied the effect of chlorophyll levels on the oxidative quality of rapeseed oil. The authors indicated that high level of chlorophyll residues promoted oxidative reactions by photo-oxidation, hence methods to reduce chlorophyll in rapeseed oil or storage in dark room must be considered. High chlorophyll content also increased the peroxide value of rapeseed oil when exposed to light while slight changes in peroxide value of oil stored in the dark regardless if chlorophyll is added (Li et al. 2018). Insignificant amount of chlorophyll found in flaxseed oil therefore contributed to oxidative stability. Chlorophyll is considered a photosensitizer which absorbs light energy to form excited singlet state and then afterward converted to excited triplet state. This excited triplet state can react easily with unsaturated fatty acids and abstract a hydrogen which trigger the initiation step of lipid oxidation (Mc Clements and Decker 2008).

4 CONCLUSIONS

Oxidative stability of the CL and NCL microencapsulated flaxseed oil materials was dependent on the RHs during storage. Increasing the humidity up to 75% enhanced the oxidative stability of both CL and NCL spray dried emulsions as exhibited in structural changes from powdery to sticky form. At moist conditions, cavities rearranged in between powder particles and oxygen diffusion was therefore limited. At lower RHs (0%, 11%, 33%) oxidation of microencapsulated oils occurred in SL and TL. Surface oils in CL microencapsulated oils were very susceptible to oxidation at the driest conditions (0% RH) as demonstrated by high PVs at the end of storage. The greater surface area of powder particles and cracking of matrix promoted oxygen diffusion at the driest storage at 0%RH; hence oxidation rate was high. However, the total lipids of CL and NCL dried emulsions were oxidatively unstable at intermediate moisture (33% RH), while both powders were stable at 54% and 75% RHs, which means that the findings of surface lipids and total lipids were different. Instability of CL and NCL spray dried emulsions was also apparent at intermediate moisture condition (33% RH), in which propanal peak areas were highest. However, hexanal peak areas correlated more with peroxide values giving the highest which was found at 0%RH in CL and 11%RH in NCL. Thus, there were differences of the results on oxidative stability among different materials and storage conditions. High amount of ALA and γ-tocopherol were also measured from both microencapsulated flaxseed oils which also decreased insignificantly through storage time and under different moisture conditions. Overall, only the PVs and secondary oxidation products were significantly affected by moisture induced changes on the interfacial and matrix properties of CL and NCL microencapsulated oils. The ALA and γ-tocopherol contents of microencapsulated oils had only slight differences and losses after storage at different timepoints and RHs. The discrepancy of results in some chemical properties were partly due to difficulty in extractions of SL and TL from microencapsulated oils.

The bulk flaxseed oil was stable against oxidation throughout the storage period. The antioxidant protective effect of γ-tocopherol, phenolic compounds, and carotenoids in FSO contributed to the oxidative stability of the oils. The reduction of γ-tocopherol upon storage correlated quite well with high PVs at 0%RH for free oils. This contrasts with the fatty acid decomposition of CL and NCL surface lipids which were noticeable at 75%RH at the end of storage, this contradictory result can also be due to difficulties in extraction. The FSO was

unpredictably stable throughout the storage experiment which also made it difficult to justify and evaluate the results between the microencapsulated oils and the bulk FSO.

This study showed that microencapsulation and interfacial protein crosslinking of microencapsulated FSO were also suitable for specific timepoints in the preservation of sensitive oils containing high concentration of PUFAs. Due to inconsistent and unexpected results from the bulk FSO and microencapsulated oils, the following recommendations were made for future studies: (1) Na-caseinate cross-linking as interface and maltodextrin as matrix can be utilized in the microencapsulation of other unstable and oxidizing oils; (2) optimization of microencapsulation efficiency of FSO; (3) the chemical and physical characterization of microencapsulated FSO can be useful in the study of other oxidizing oils (4) the use of native FSO instead of commercial FSO for further studies in the optimization of microencapsulation process parameters and oxidative stability storage (5) the exploitation on the use of microencapsulated FSO in other industrial food product applications.

REFERENCES

Adachi S, Imaoka H, Matsuno R. 2003. Preparation of W/O/W microcapsules by spray-drying. Proceedings of the European Drying Symposium. Heraklion-Crete, Greece. 296-301.

Andersen AB, Risbo J, Andersen ML, Skibsted LH.2000 Oxygen permeation through an oil-encapsulating glassy food matrix studied by ESR line broadening using a nitroxyl spin probe. Food Chemistry 70(4): 499–508

Belitz HD, Grosch W, Schieberle PS. 2004. Food Chemistry. 3rd edition. Heidelberg, Germany: Spinger – Verlag. 1070 p.

Buchert J, Cura DE, Ma H, Gasparetti C, Monogioudi E, Faccio G, Mattinen M, Boer H, Partanen R, Selinheimo E, Lantto R, and Kruus K. 2010. Crosslinking food proteins for improved functionality. Annual Review of Food Science and Technology 1:113-138.

Buckton G, Chidavaenzi O, Koosha F. 2002. The effect of spray-drying feed temperature and subsequent crystallization conditions on the physical form of lactose. AAPS PharmSciTech 3(4): 37

Carneiro HCF, Tonon RV. Grosso CRF, Hubinger MD. 2013. Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. Journal of Food Engineering 115: 443-451

Carter JF. 1993. Potential of flaxseed and flaxseed oil in baked goods and other products in human nutrition. Cereal Foods World 38: 753-759.

Christie WW. 1989. Gas chromatography and lipids: A practical guide. 1st edition. Dundee, Scotland: The Oily press Ltd. 307 p.

Coultate T. 2009. Food: The Chemistry of its components. 5th edition. Cambridge, UK: The Royal Society of Chemistry. 501 p.

Cunnane SC, Thompson LU. 1995. Flaxseed in Human Nutrition. AOCS Press, Champaign II.

Damerau A, Moisio T, Partanen R, Forssell P, Lampi AM, Piironen V. 2014. Interfacial protein engineering for spray-dried emulsions -Part II: Oxidative stability. Journal of Food Chemistry 144:57-64.

Dickinson E**.** 1997**.** Enzymic crosslinking as a tool for food colloid rheology control and interfacial stabilization. Trends Food Sci. Technol. 8:334–39

Drusch S, Rätzke K, Shaikh MQ, Serfert Y, Steckel H, Scampicchio M. 2009. Differences in free volume elements of the carrier matrix affect the stability of microencapsulated lipophilic food ingredients. Food Biophysics 4(1): 42−48.

Elversson J, Millqvist-Fureby A. 2005. Particle Size and Density in Spray Drying—Effects of Carbohydrate Properties. Journal of Pharmaceutical Sciences 94: 2049-2060.

Færgemand M, Otte J, and Qvist KB. 1998. Emulsifying properties of milk proteins crosslinked with microbial transglutaminase. Int. Dairy Journal 8:715-723.

Frankel EN. 1998. Lipid Oxidation. Dundee, Scotland: The Oily Press Ltd. 303 p.

Gauvin WH, Katta S. 1976. Basic concepts of spray dryer design. AIChe Journal 22(4):713-724

Gharsallaoui A, Roudaut G, Chambin O, Voilley A, Saurel R. 2007. Applications of spray-drying in microencapsulation of food ingredients: An overview. J. Food Research International 40:1107–1121.

Gouin S. 2004. Microencapsulation: Industrial appraisal of existing technologies and trends. Trends in Food Science and Technology 15:330-347.

Goyal A, Sharma V, Upadhyay N, Gill S, Sihag M. 2014. Flax and flaxseed oil: an ancient medicine & modern functional food. J Food Sci Technol 51(9):1633–1653.

Green AG, Dribnenki JCP. 1994. Linola – a mew premium polyunsaturated oil. Lipid Technology 6:29-33.

Gunstone FD. 1996. Practical applications of oils and fats. In: Fatty Acid and Lipid Chemistry. Spinger, Boston, MA. pp. 229-243.

Hardas N, Danviriyakul S, Foley JL, NAwar WW, Chinachoti O. 2002. Effect of relative humidity on the oxidative and physical stability of encapsulated milk fat. Journal of American Oil Chemist Society 79(2): 151-158.

Hasiewicz-Derkacz K, Kulma A, Czuj T,4, Prescha A , Żuk M, Grajzer M , Łukaszewicz, Szopa J. 2015. Natural phenolics greatly increase flax (Linum usitatissimum) oil stability. BMC Biotechnology 15:62-76.

Haumann BF. 1990. Low-linolenic flax: variaytion on familiar oilseed. American Oil Chemists' Society 1:934-941.

Herchi W. Sakouhi F. Boukhchina S. Kallel H. Pepe C. 2011. Changes in Fatty Acids, Tocochromanols, Carotenoids and Chlorophylls Content During Flaxseed Development. Journal of American Oil Chem. Soc. 88:1011–1017

Herchi W, Román DA, Boukhchina S, Kallel H, Carretero AS, Gutierrez AF. 2012. A review of the methods used in the determination of flaxseed components. African Journal of Biotechnology 11(4): 724-731.

Hornero-Mendez D, Perez-Galvez A, Minguez-Mosquera MI. 2001. A rapid spectrophotometric method for the determination of peroxide value in food lipids with high carotenoid. J Am Oil Chem Soc 78(11):1151-5.

Jafari SM, Assadpoor E, He Y, Bhandari B. 2008. Encapsulation Efficiency of food flavours and oils during spray drying. Drying Technology 26: 816–835.

Jaros D**,** Pätzold J**,** Schwarzenbotz U**,** Rohm H**.** 2006b**.** Small and large deformation rheology of acid gels from transglutaminase treated milks. FOBI 1:124–32

Jayasundera M, Adhikari B, Aldred P, Ghandi A. 2009. Surface modification of spray dried food and emulsion powders with surface-active proteins. A review. J Food Eng 93:266-277.

Kahl JL, Artz, E, Schanus EG. 1988. Effects of relative humidity on lipid autoxidation in a model system. Lipids 23 (4): 275-279.

Klinkesorn U, Sophanodora P, Chinachoti P, Mc Clements DJ, Decker EA. 2005. Stability of spray-dried tuna oil emulsions encapsulated with two-layered interfacial membranes. Journal of Agricultural and Food Chemistry 53 (21): 8365-8371.

Kolb B, Ettre L. 1997.Static Headspace – Gas Chromatography. Theory and Practice, 1st edition. USA: Wiley-VCH, Inc. 298 p.

Labuza TP, Heidelbaugh M, Karel M. 1971. Oxidation at intermediate moisture contents. Journal of the American Oil Chemists' Society, 48, 86–90.

Labuza TP, McNally L, Gallagher D, Hawkes J, Hurtado D. 1972. Stability of intermediate moisture foods. 1. Lipid oxidation. Journal of Food Science, 37, 154–159

Labuza TP. 1980. The effect of water activity on reaction kinetics of food deterioration. Food Tech. 34(4): 36-41.

Lampi AM. 2009. EK 331 Research Methods course materials. Department of Applied Chemistry and Microbiology. University of Helsinki, Finland. 15 p.

Lehtonen M, Lampi AM, Ollilainen V, Struijs K, Piironen V. 2011. The role of acyl moiety in the formation and reactions of steryl ester hydroperoxides. European Food Research and Technology, 233(1), 51–61.

Lewandowicz G, Prochaska K, Grajek W, Krzyżaniak W, Majchrzak A, and Ciapa T, 2005. Functional properties of maltodextrins in emulsion systems. Żywność. Technologia. Jakość. 42: 35–47.

Li X, Yang R, Lv C, Chen L, Zhang L, Ding X. Zhang W, Zhang Q, Hu C, Li P. 2019. Effect of chlorophyll on lipid oxidation of rapeseed oil. European Journal of Lipid Science and Technology 121 (1800078) :1-4.

Ma H, Forssell P, Kylli P, Lampi AM, Buchert J, Boer H, Partanen R. 2012. Transglutaminase catalyzed cross-linking of sodium caseinate improves oxidative stability of flaxseed oil emulsion. J. Agric. Food Chem. 60: 6223−6229

McClements DJ and Decker EA. 2008. Lipids. In: Damodaran S, Parlin KL, Fennema OR. Food Chemistry. 4th edition. Boca Raton, London, New York: Taylor and Francis Group, LLC. p 156-216 p.

Minemoto Y, Hakamata K, Adachi S, Matsuno R. 2002. Oxidation of linoleic acid encapsulated with gum arabic or maltodextrin by spray drying. Journal of Microencapsulation 19(2): 181–189.

Moisio T, Damerau A, Lampi AM, Piironen V, Forssell P, Partanen R. 2014. Interfacial protein engineering for spray–dried emulsions – Part I: Effects on protein distribution and physical properties. Journal of Food Chemistry 144: 50-56

Motoki M, Seguro K. 1998. Transglutaminase and its use for food processing. Trends Food Sci. Technol. 9:204–10

Partanen R, Yoshii H, Kallio H, Yang B, Forssell P. 2002 Encapsulation of sea buckthorn kernel oil in modified starches. JAOCS 79(3): 219-223.

Partanen R, Hakala P, Sjövall O, Kallio H, Forssell P. 2005. Effect of relative humidity on the oxidative stability of microencapsulated sea buckthorn seed oil. Journal of Food Science 70(1): E37−E43.

Partanen R, Raula J, Seppänen R, Buchert J, Kauppinen E, Forssell P. 2008. Effect of relative humidity on oxidation of flaxseed oil in spray dried whey protein emulsions. Journal of Agricultural and Food Chemistry 56(14): 5717−5722.

Pegg RB, Shahidi F. 2007. Encapsulation, stabilization, and controlled release of food ingredients and bioactives. In: Handbook of Food Preservation. 2nd edition. p. 509-568.

Prevc T, Levart A, Cigić IK, Salobir J, Ulrih NP, Cigić B. 2015. Rapid Estimation of tocopherol content in linseed and sunflower oils - Reactivity and assay. Journal of Molecules 20: 14777-14790.

Rubilar M, Morales E, Contreras K, Ceballos C, Acevedo F, Villarroel M, Shene C. 2012. Development of a soup powder enriched with microencapsulated linseed oil as a source of omega-3 fatty acids. Eur. J. Lipid Sci. Technol. 114: 423–433

Sakamoto H, Yamazaki K, Kaga C, Yamamoto Y, Ito R, Kurosawa Y. 1996. Strength enhancement by addition of microbial transglutaminase during Chinese noodle processing. Nippon Shokuhin Kagaku Kogaku Kaishi 43:598–602

Schaich KM, Karel M. 1976. Free radical reactions of peroxidizing lipids with amino acids and proteins: An ESR study. Lipids 11(5): 329-400.

Schaich KM. 2008. Co-oxidation of proteins by oxidizing lipids. In A. Kamal-Eldin and DB Min (Ed) Lipid oxidation pathways, Volume 2. pp181-272. Urbana, IL, USA: AOCS Press.

Schwartz H, Olllilainen V, Piironen V, Lampi AM. 2008. Tocopherol, tocotrienol, and plant sterol contents of vegetable oils and industrial fats. Journal of Food Composition and Analysis 21(2):152-161.

Schwartz SJ, von Elbe JH, Giusti MM. 2008. Colorants. In: Damodaran S, Parlin KL, Fennema OR. Food Chemistry. 4th edition. Boca Raton, London, New York: Taylor and Francis Group, LLC. pp 572-632.

Shahidi F, Han XQ. 1993. Encapsulation of food ingredients. Critical Review in Food Science and Nutrition 33: 501–547.

Shahidi F. de Camargo AC. 2016. Tocopherols and Tocotrienols in Common and Emerging Dietary Sources: Occurrence, Applications, and Health Benefits. Int. J. Mol. Sci. 17:1745-1774.

Soottitantawat A, Yoshii H, Furuta T, Ohkawara M, Linko P. 2003 Microencapsulation by spray drying: Influence of emulsion size on the retention of volatile compounds. Journal of Food Science 68(7): 2256-2262

Soupas L, Huikko L, Lampi AM, Piironen V. 2005. Esterification affects phytosterol oxidation. European Journal of Lipid Science and Technology 107(2): 107-118.

Tarpila A, Wennberg T, Tarpila S. 2005. Flaxseed as a functional food. Curr Top Nutraceuticals Res 3(3):167–188

Tonon RV, Grosso CRF, Hubinger MD. 2011. Influence of emulsion composition and inlet air temperature on the microencapsulation of flaxseed oil by spray drying. Food Research International 44: 282–289.

Tonon RV, Pedro RB, Grosso CRF, Hubinger MD. 2012. Microencapsulation of flaxseed oil by spray drying: effect of oil load and type of wall material. Drying Technology 30: 1491–1501.

Traore F, Meunier JC. 1992. Crosslinking Activity of Placental F XIIIa on Whey Proteins and Caseins. Journal of agricultural and food chemistry 40(3): 399–402.

Tuberoso CIG, Kowalczyk A, Sarritzu E, Cabras P. 2007. Determination of antioxidant compounds and antioxidant activity in commercial oilseeds for food use. Food Chem. 103:1494–501.

Vaisey-Genser M. 1994. Flaxseed: Health, Nutrition and Functionality. The Flax Council of Canada, Winnipeg.

Vega-Mercado H, Gongora-Nieto M, Barbosa-Canovas G. 2001. Advances in dehydration of foods. Journal of Food Engineering 49:271–289.

Vehring R. 2008. Pharmaceutical Particle Engineering via Spray Drying. Pharmaceutical Research 25:999-1022.

Vega C. and Roos Y.H. 2006 Spray-dried dairy and dairy-like emulsions - Compositional considerations. Journal of Dairy Science 899:383-401.

Velasco PJ, Dobarganes C, Ruiz GM. 2003. Variables affecting lipid oxidation in dried microencapsulated oils. Grasas y Aceites 54: 304-314.

Velasco J, Holgado F, Dobarganes C, Márquez-Ruiz G. 2009. Influence of relative humidity on oxidation of the free and encapsulated oil fractions in freeze-dried microencapsulated oils. Food Research International 42: 1492-1500.

Vieira S. Zhang G. Decker EA. 2017. Biological Implications of Lipid Oxidation Products. J Am Oil Chem Soc DOI 10.1007/s11746.

Walstra P, Vliet TV. 2008. Dispersed systems: Basic considerations. In: Damodaran S, Parlin KL, Fennema OR. Food Chemistry. 4th edition. Boca Raton, London, New York: Taylor and Francis Group, LLC. p 783-847.

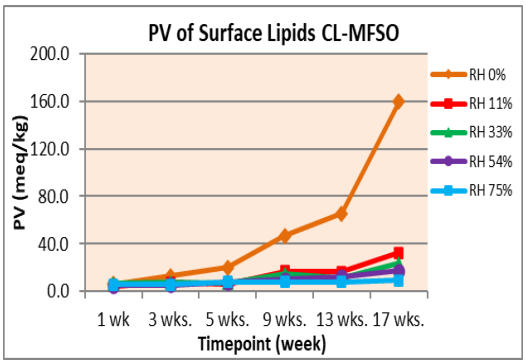
Waraho T, McClements DJ, Decker EA. 2011. Mechanisms of lipid oxidation in food dispersions. Trends in Food Science & Technology 22:3-13

Yu X, Tang Y, Liu P, Xiao L, Liu L, Shen R L, Yao P. 2017. Flaxseed oil alleviates chronic HFD-Induced insulin resistance through remodeling lipid homeostasis in obese adipose tissue. Journal of Agriculture and Food Chemistry, 65(44).

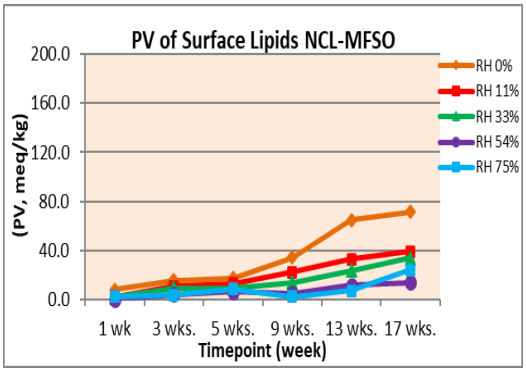
Zhu Y**,** Bol J**,** Rinzema A**,** Tramper J**,** Wijngaards G**.** 1999**.** Transglutaminase as a potential tool in developing novel protein foods. Agro-Food Hi-Tech. Jan./Feb.:8–10

Appendix 1. Peroxide Values (meq/kg) of CL-MFSO, NCL-MFSO, and FSO

PV of CL-MFSO in Surface Lipids (meq/kg)						
Relative Humidity (%)	1 wk	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	6.4	13.0	20.0	46.8	65.3	159.7
RH 11%	4.3	7.7	6.2	16.7	16.6	32.7
RH 33%	6.7	7.4	7.2	14.6	11.5	23.6
RH 54%	4.7	5.5	7.5	10.2	12.2	17.5
RH 75%	5.5	5.5	7.9	7.8	7.9	9.1

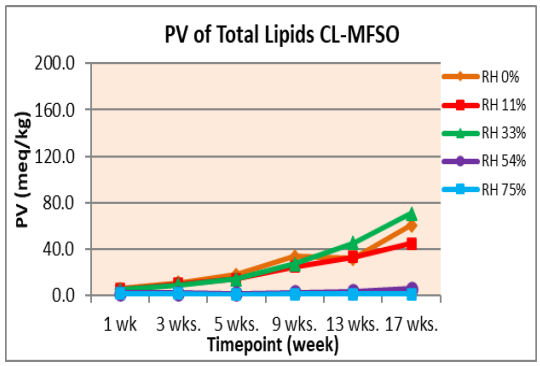


PV of NCL-MFSO in Surface Lipids (meq/kg)						
Relative Humidity (%)	1 wk	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	8.2	15.5	17.4	34.1	64.6	71.5
RH 11%	2.2	10.6	13.1	22.1	32.7	39.0
RH 33%	1.7	9.3	9.0	13.8	23.6	33.7
RH 54%	0.2	3.9	6.2	4.8	11.5	13.6
RH 75%	2.2	3.6	8.3	2.1	7.2	23.9

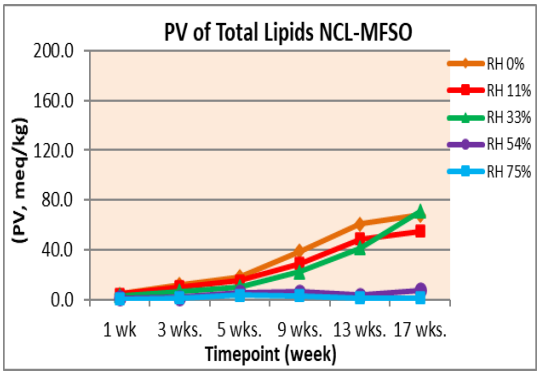


Appendix 1.1 Peroxide Values (meq/kg) of Surface Lipids CL-MFSO and NCL-MFSO

PV of CL-MFSO in Total Lipids (meq/kg)						
Relative Humidity (%)	1 wk	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	5.9	10.9	18.0	33.6	32.1	60.7
RH 11%	5.7	10.3	14.4	25.0	33.4	45.0
RH 33%	5.2	9.3	14.2	27.3	45.3	70.7
RH 54%	2.3	2.2	1.6	2.8	3.6	6.0
RH 75%	1.8	1.8	1.0	1.4	1.2	1.1

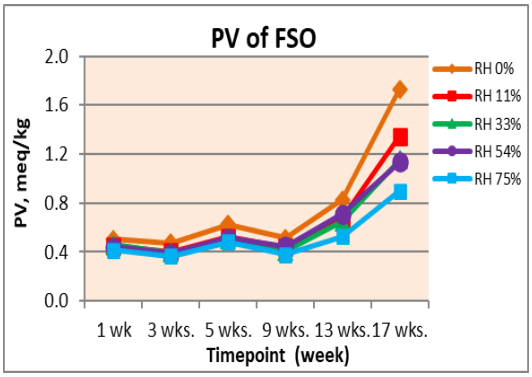


PV of NCL-MFSO in Total Lipids (meq/kg)						
Relative Humidity (%)	1 wk	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	4.7	12.0	18.2	39.2	60.7	67.8
RH 11%	4.4	10.4	15.1	29.0	48.6	54.9
RH 33%	3.4	6.6	10.5	22.6	41.6	70.9
RH 54%	1.3	1.8	5.7	6.4	3.9	7.5
RH 75%	0.7	1.4	3.6	3.4	1.3	1.5



Appendix 1.2 Peroxide Values (meq/kg) of Total Lipids CL-MFSO and NCL-MFSO

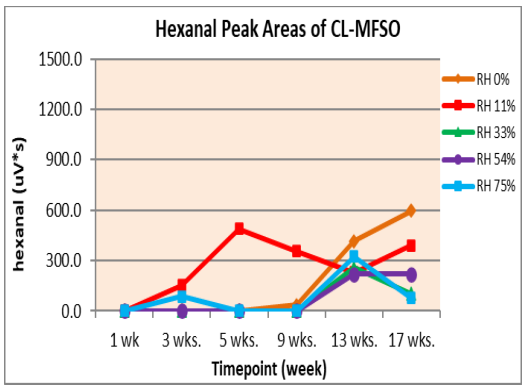
	FSO - PV (meq/kg)					
Relative Humidity (%)	1 wk	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	0.5	0.5	0.6	0.5	0.8	1.7
RH 11%	0.5	0.4	0.5	0.4	0.7	1.3
RH 33%	0.5	0.4	0.5	0.4	0.6	1.2
RH 54%	0.4	0.4	0.5	0.4	0.7	1.1
RH 75%	0.4	0.4	0.5	0.4	0.5	0.9



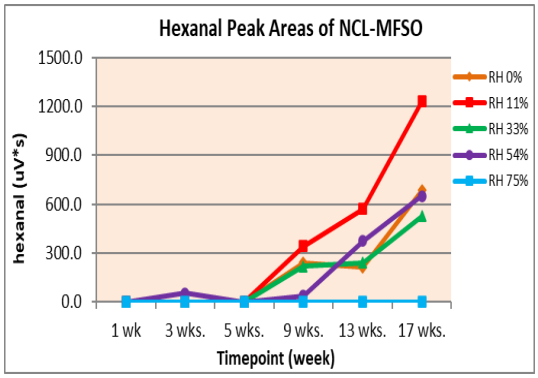
Appendix 1.3 Peroxide Values (meq/kg) of FSO

Appendix 2. Hexanal Peak Areas (uV*s) of CL-MSO, NCL-MFSO, and FSO

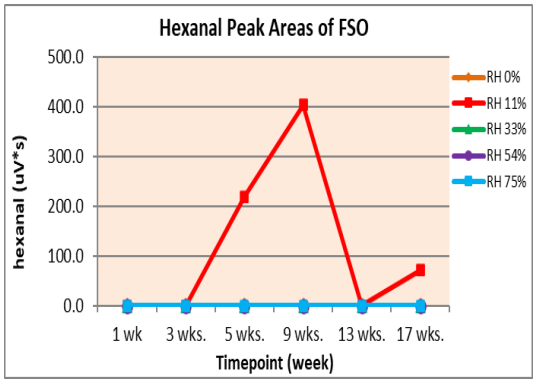
	Hexanal Peak Areas of CL-MFSO (uV*s)					
Relative Humidity (%)	1 wk	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	0.0	0.0	0.0	34.8	415.8	596.3
RH 11%	0.0	152.9	489.3	354.7	229.2	389.5
RH 33%	0.0	0.0	0.0	0.0	257.1	103.2
RH 54%	0.0	0.0	0.0	0.0	220.3	218.4
RH 75%	0.0	86.5	0.0	0.0	323.8	78.1



	Hexanal Peak Areas of NCL-MFSO (uV*s)					
Relative Humidity (%)	1 wk	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	0.0	0.0	0.0	241.2	212.4	685.0
RH 11%	0.0	0.0	0.0	341.6	571.9	1227.7
RH 33%	0.0	0.0	0.0	218.0	240.7	527.3
RH 54%	0.0	53.0	0.0	39.1	373.4	650.1
RH 75%	0.0	0.0	0.0	0.0	0.0	0.0

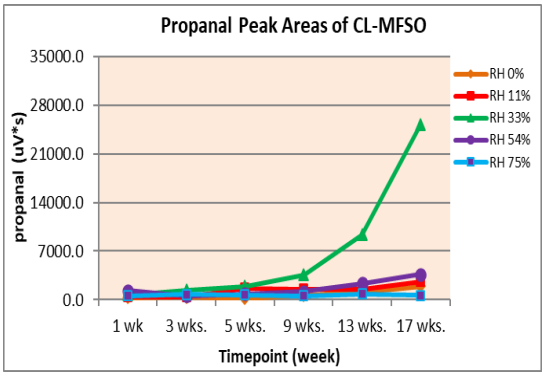


	Hexanal Peak Areas of FSO (uV*s)					
Relative Humidity (%)	1 wk	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	0.0	0.0	0.0	0.0	0.0	0.0
RH 11%	0.0	0.0	219.9	403.8	0.0	71.8
RH 33%	0.0	0.0	0.0	0.0	0.0	0.0
RH 54%	0.0	0.0	0.0	0.0	0.0	0.0
RH 75%	0.0	0.0	0.0	0.0	0.0	0.0

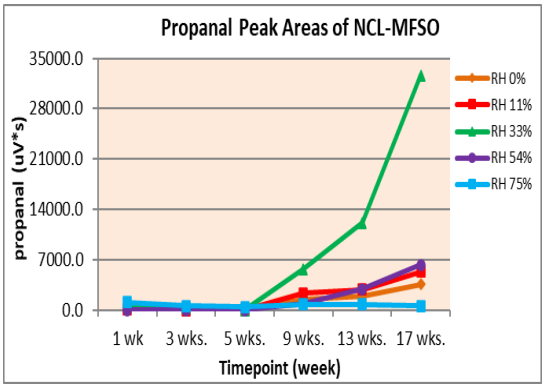


Appendix 3. Propanal Peak Areas (uV*s) of CL-MFSO, NCL-MFSO, and FSO

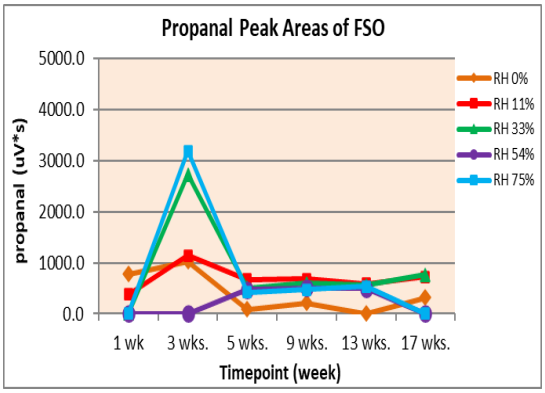
	Propanal Peak Areas of CL-MFSO (uV*s)					
Relative Humidity (%)	1 wk	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	289.2	333.4	185.9	665.6	1100.3	1970.9
RH 11%	436.0	456.8	1683.3	1506.7	1480.9	2664.3
RH 33%	677.1	1430.9	1914.4	3574.8	9383.9	25264.2
RH 54%	1331.9	598.0	813.8	1180.7	2345.4	3659.2
RH 75%	571.8	784.3	696.9	584.6	879.4	631.3



	Propanal Peak Areas of NCL-MFSO (uV*s)					
Relative Humidity (%)	1 wk	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	356.4	22.6	0.0	1522.5	1870.4	3576.6
RH 11%	46.2	0.0	0.0	2351.2	2819.8	5313.2
RH 33%	651.0	176.0	0.0	5672.9	12151.3	32597.4
RH 54%	0.0	0.0	0.0	860.4	2885.3	6287.7
RH 75%	1071.1	557.3	452.5	778.0	781.1	530.7

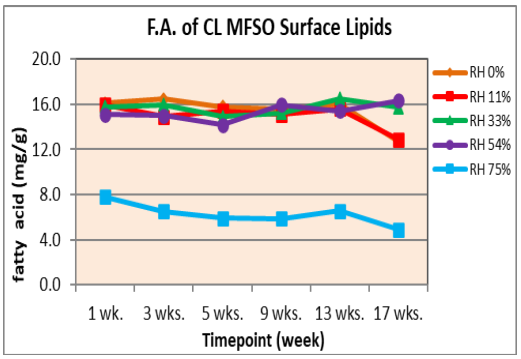


	Propanal Peak Areas of FSO(uV*s)					
Relative Humidity (%)	1 wk	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	778.3	1031.6	88.0	209.2	0.0	311.4
RH 11%	376.7	1146.5	667.8	676.2	586.0	722.1
RH 33%	0.0	2734.1	505.6	596.1	556.0	761.0
RH 54%	0.0	0.0	467.5	500.5	496.3	0.0
RH 75%	0.0	3189.8	423.9	473.0	535.9	0.0

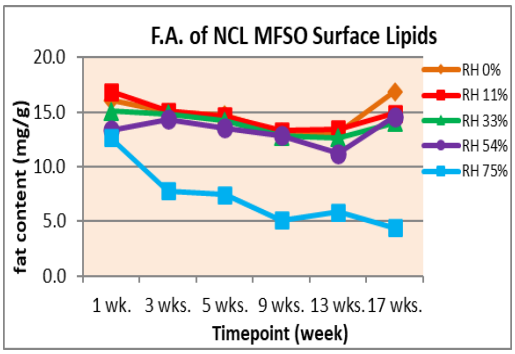


Appendix 4. Fatty Acid Content (mg/g) of CL-MFSO, NCL-MFSO, and FSO

	Fatty Acid (mg/g) of CL-MFSO in Surface Lipids					
Relative Humidity (%)	1 wk.	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	16.1	16.5	15.8	15.6	16.1	12.8
RH 11%	15.9	14.9	15.4	15.1	15.6	12.9
RH 33%	15.8	15.9	14.9	15.2	16.5	15.8
RH 54%	15.1	15.0	14.2	16.0	15.4	16.3
RH 75%	7.8	6.5	5.9	5.9	6.6	4.9

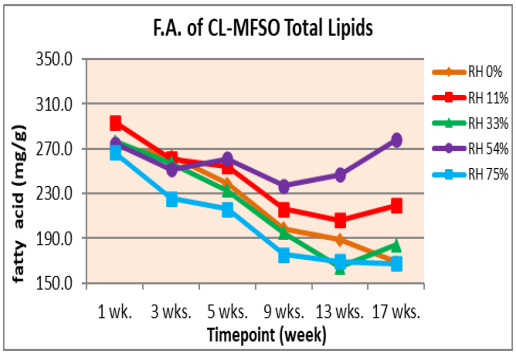


	Fatty Acid (mg/g) of NCL-MFSO in Surface Lipids					
Relative Humidity (%)	1 wk.	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	16.1	15.0	14.7	13.2	13.1	16.9
RH 11%	16.9	15.1	14.7	13.3	13.5	14.9
RH 33%	15.1	14.8	14.2	12.8	12.6	14.0
RH 54%	13.4	14.3	13.5	12.8	11.2	14.6
RH 75%	12.7	7.8	7.4	5.1	5.8	4.4

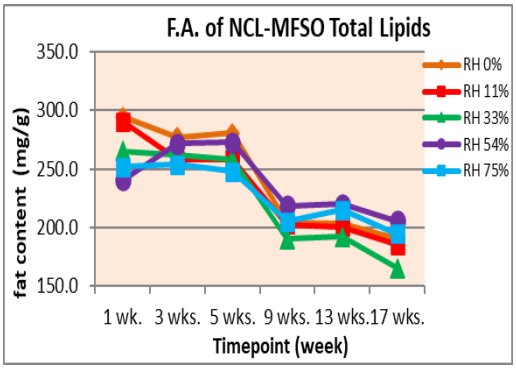


Appendix 4.1 Fatty Acid Content of Surface Lipids CL-MFSO and NCL-MFSO

	Fatty Acid (mg/g)of CL-MFSO in Total Lipids					
Relative Humidity (%)	1 wk.	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	272.1	263.4	238.7	198.6	188.6	168.7
RH 11%	293.4	261.1	254.3	216.0	206.0	219.5
RH 33%	276.6	257.0	232.7	194.9	164.3	184.0
RH 54%	274.5	251.7	260.9	236.9	247.0	278.2
RH 75%	266.4	225.5	216.2	175.4	169.3	167.2

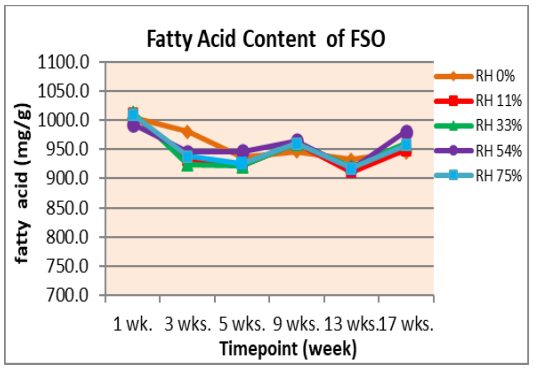


	Fatty Acid (mg/g) of NCL-MFSO in Total Lipids					
Relative Humidity (%)	1 wk.	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	294.6	277.0	280.7	204.4	203.3	190.1
RH 11%	290.6	257.8	258.5	202.6	200.4	185.0
RH 33%	265.4	262.0	258.4	189.8	192.2	165.3
RH 54%	240.4	271.6	272.7	218.4	219.7	205.3
RH 75%	251.7	253.8	247.6	204.7	214.9	194.5



Appendix 4.2 Fatty Acid Content of Total Lipids CL-MFSO and NCL-MFSO

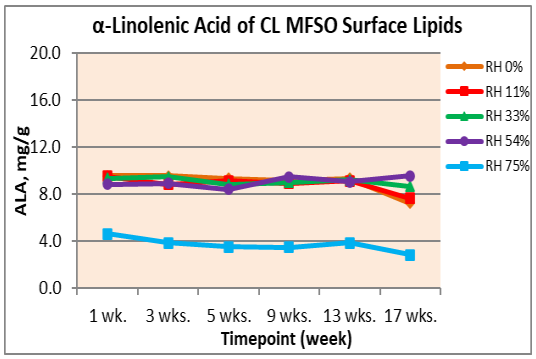
	Fatty Acid Content of FSO (mg/g)					
Relative Humidity (%)	1 wk.	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	1004.0	980.3	935.7	945.7	932.0	945.0
RH 11%	1010.2	932.5	921.6	960.0	911.4	949.0
RH 33%	1013.3	923.9	920.9	960.2	920.4	959.9
RH 54%	992.8	944.8	946.3	964.3	916.5	979.7
RH 75%	1009.1	937.5	926.1	960.0	917.4	956.8



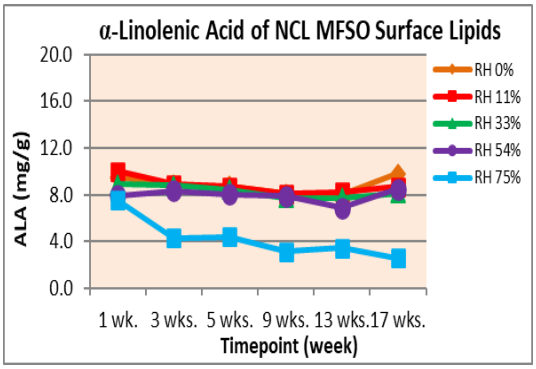
Appendix 4.3 Fatty Acid Content of FSO

Appendix 5. α-Linoleic content (mg/g) of CL-MFSO, NCL-MFSO, and FSO

	α-Linolenic Acid (mg/g) of CL-MFSO in Surface Lipids					
Relative Humidity (%)	1 wk.	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	9.6	9.6	9.4	9.2	9.3	7.2
RH 11%	9.5	8.8	9.2	8.9	9.2	7.6
RH 33%	9.4	9.5	8.8	9.0	9.2	8.7
RH 54%	8.8	8.9	8.4	9.5	9.0	9.6
RH 75%	4.6	3.9	3.5	3.5	3.9	2.9

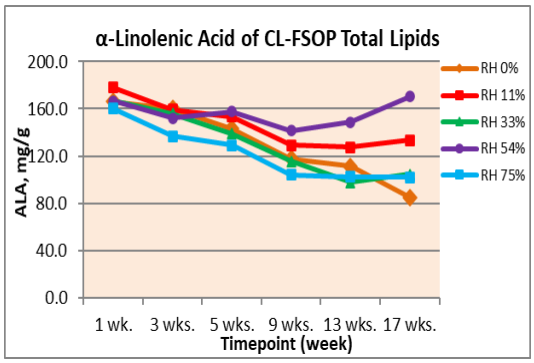


	α-Linolenic Acid (mg/g) of NCL-MFSO in Surface Lipids					
Relative Humidity (%)	1 wk.	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	9.5	8.8	8.7	8.1	8.0	9.8
RH 11%	10.0	8.9	8.7	8.1	8.2	8.7
RH 33%	8.9	8.8	8.4	7.7	7.7	8.1
RH 54%	8.0	8.3	8.0	7.9	6.9	8.5
RH 75%	7.5	4.3	4.4	3.2	3.5	2.6

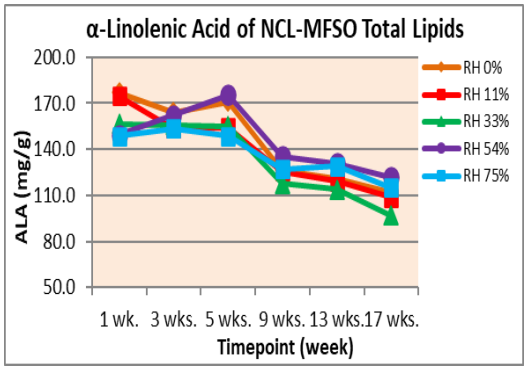


Appendix 5.1 α-Linoleic Content of Surface lipids CL-MFSO and NCL-MFSO

	α-Linolenic Acid (mg/kg) of CL-MFSO in Total Lipids					
Relative Humidity (%)	1 wk.	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	166.1	160.3	143.3	117.7	111.6	85.2
RH 11%	177.8	159.3	153.2	129.3	127.5	133.5
RH 33%	166.6	155.8	139.0	115.6	97.7	104.5
RH 54%	166.5	152.3	157.5	141.6	148.9	170.6
RH 75%	160.4	137.1	129.3	104.0	102.6	102.2

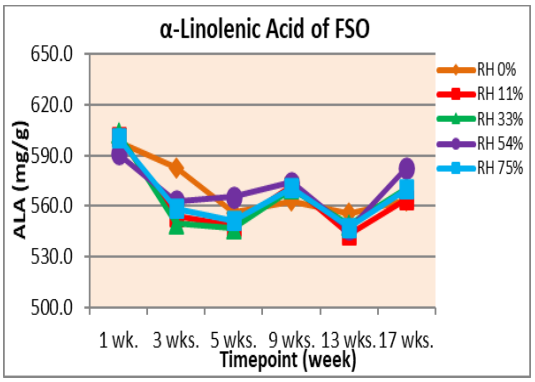


	α-Linolenic Acid (mg/g) of NCL-MFSO in Total Lipids					
Relative Humidity (%)	1 wk.	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	176.8	164.0	170.7	126.2	120.6	111.5
RH 11%	174.5	153.5	154.1	125.6	119.0	108.7
RH 33%	156.7	155.7	155.1	117.7	113.8	96.8
RH 54%	149.3	162.0	175.4	135.5	131.0	121.8
RH 75%	148.6	153.7	148.4	127.2	129.0	115.1



Appendix 5.2 α-Linoleic Content of Total lipids CL-MFSO and NCL-MFSO

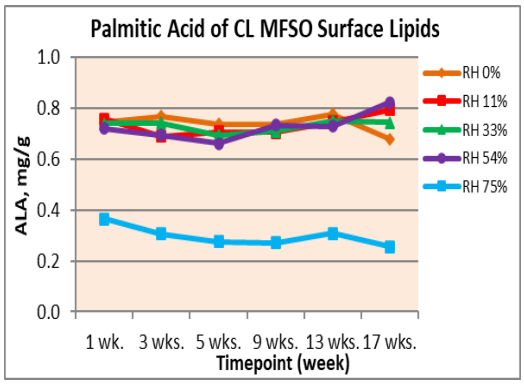
	α-Linolenic Acid Content (mg/g) of FSO					
Relative Humidity (%)	1 wk.	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	597.8	582.6	555.9	562.8	555.6	563.4
RH 11%	601.5	553.9	548.0	572.0	543.0	563.9
RH 33%	603.3	549.6	546.9	570.2	548.9	570.9
RH 54%	590.9	562.8	565.3	574.0	546.3	582.8
RH 75%	600.3	558.6	551.5	570.9	547.3	570.0



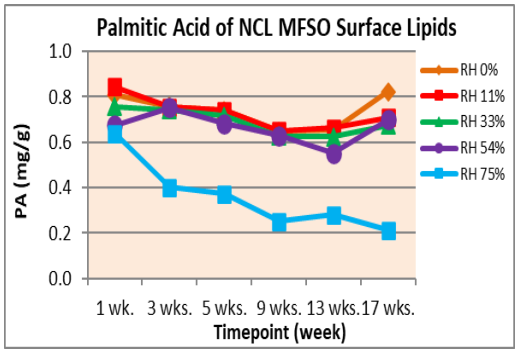
Appendix 5.3 α-Linoleic Content of FSO

Appendix 6. Palmitic Acid Content (mg/g) of CL-MFSO, NCL-MFSO, and FSO

	Palmitic Acid (mg/g) of CL-MFSO in Surface Lipids					
Relative Humidity (%)	1 wk.	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	0.7	0.8	0.7	0.7	0.8	0.7
RH 11%	0.8	0.7	0.7	0.7	0.7	0.8
RH 33%	0.7	0.7	0.7	0.7	0.8	0.7
RH 54%	0.7	0.7	0.7	0.7	0.7	0.8
RH 75%	0.4	0.3	0.3	0.3	0.3	0.3

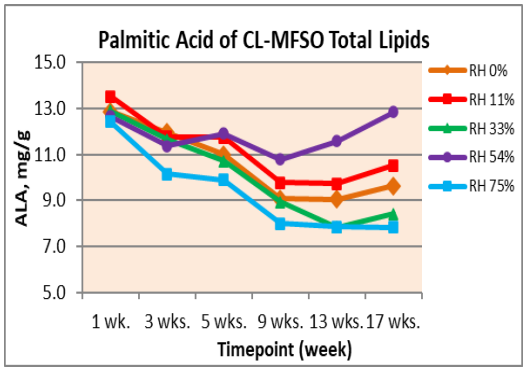


	Palmitic Acid (mg/g) of NCL-MFSO in Surface Lipids					
Relative Humidity (%)	1 wk.	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	0.8	0.8	0.7	0.6	0.7	0.8
RH 11%	0.8	0.8	0.7	0.7	0.7	0.7
RH 33%	0.8	0.7	0.7	0.6	0.6	0.7
RH 54%	0.7	0.8	0.7	0.6	0.6	0.7
RH 75%	0.6	0.4	0.4	0.3	0.3	0.2

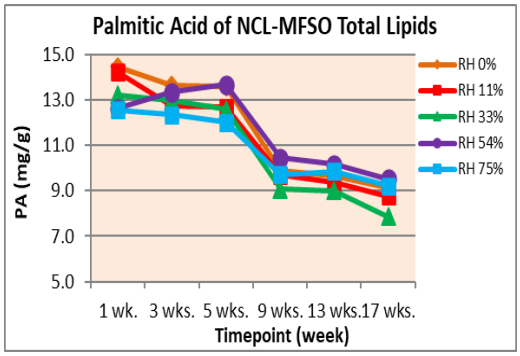


Appendix 6.1 Palmitic Acid Content of Surface Lipids of CL-MFSO and NCL-MFSO

	Palmitic Acid (mg/g) of CL-MFSO in Total Lipids					
Relative Humidity (%)	1 wk.	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	12.9	11.9	11.0	9.1	9.1	9.6
RH 11%	13.5	11.8	11.7	9.8	9.7	10.5
RH 33%	12.9	11.6	10.7	8.9	7.8	8.4
RH 54%	12.6	11.4	11.9	10.8	11.6	12.8
RH 75%	12.4	10.1	9.9	8.0	7.8	7.8

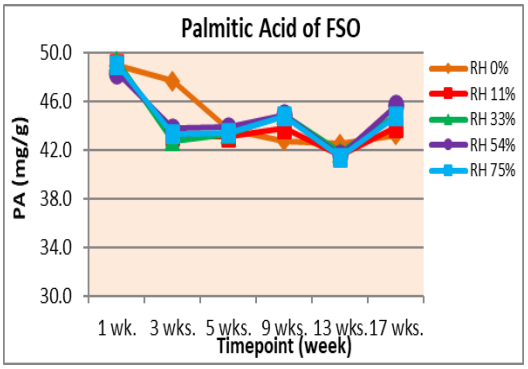


	Palmitic Acid (mg/g) of NCL-MFSO in Total Lipids					
Relative Humidity (%)	1 wk.	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	14.4	13.6	13.6	9.9	9.6	9.1
RH 11%	14.2	12.7	12.7	9.7	9.4	8.8
RH 33%	13.2	13.0	12.6	9.1	9.0	7.9
RH 54%	12.6	13.3	13.7	10.5	10.2	9.5
RH 75%	12.5	12.3	12.0	9.7	9.9	9.2



Appendix 6.2 Palmitic Acid Content of Total lipids CL-MFSO and NCL-MFSO

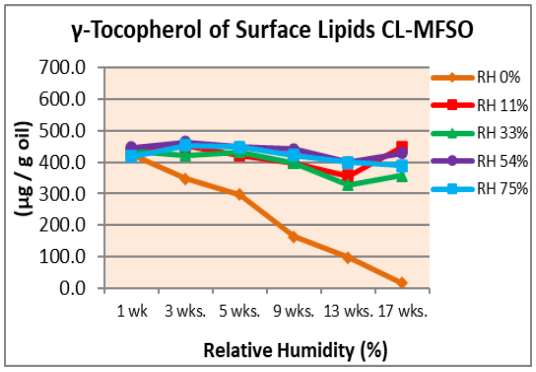
	Palmitic Acid Content (mg/g)of FSO					
Relative Humidity (%)	1 wk.	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	48.9	47.7	43.7	42.7	42.5	43.2
RH 11%	49.1	43.2	43.1	43.7	41.5	43.9
RH 33%	49.3	42.7	43.3	44.9	41.8	44.8
RH 54%	48.4	43.8	43.8	44.9	41.5	45.7
RH 75%	49.0	43.3	43.4	44.8	41.4	44.8



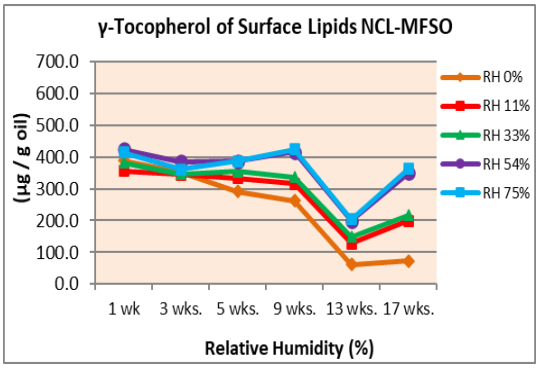
Appendix 6.3 Palmitic Acid Content of FSO

Appendix 7.1 γ-Tocopherol Content (µg/g oil) CL-MFSO, NCL-MFSO, and FSO

	γ-Tocopherol (µg/g oil)of CL-MFSO in Surface Lipids					
Relative Humidity (%)	1 wk	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	423.0	347.0	296.6	163.6	97.5	15.1
RH 11%	428.7	452.2	421.6	397.2	355.5	448.9
RH 33%	433.7	422.4	432.5	397.0	328.6	357.7
RH 54%	446.2	463.9	448.3	441.8	399.4	428.6
RH 75%	418.1	453.9	448.3	422.1	399.4	388.6

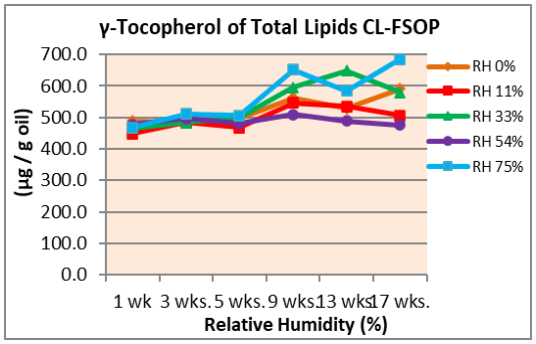


	γ-Tocopherol (µg/g oil) of NCL-MFSO in Surface Lipids					
Relative Humidity (%)	1 wk	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	389.2	351.5	291.4	261.8	61.6	72.2
RH 11%	356.1	345.4	333.5	315.6	128.5	199.8
RH 33%	382.3	346.5	354.6	336.1	149.1	217.0
RH 54%	424.5	385.8	387.5	416.6	199.4	349.6
RH 75%	415.0	361.7	387.5	423.9	203.6	362.5

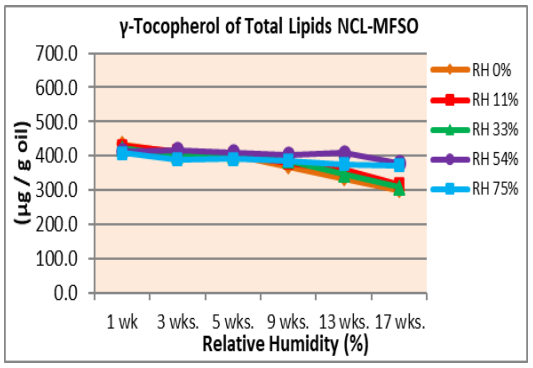


Appendix 7.1 γ-Tocopherol of Surface Lipids CL-MFSO and NCL-MFSO

	γ-Tocopherol (µg/g oil) of CL-MFSO in Total Lipids					
Relative Humidity (%)	1 wk	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	489.4	483.9	498.6	562.0	528.8	591.5
RH 11%	449.5	483.7	468.1	546.5	535.0	506.3
RH 33%	466.0	484.7	501.5	595.7	647.8	579.2
RH 54%	476.9	497.3	480.9	509.4	489.2	475.5
RH 75%	467.5	510.4	506.4	651.7	584.7	685.8

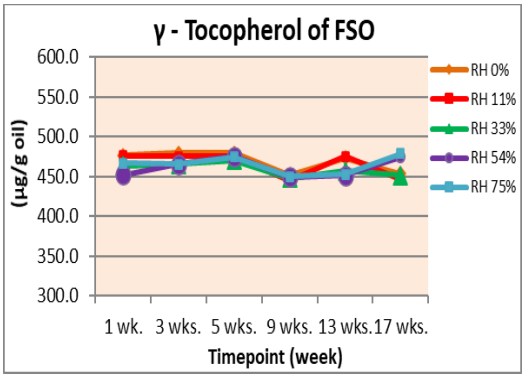


	γ-Tocopherol (µg/g oil) of NCL-MFSO in Total Lipids					
Relative Humidity (%)	1 wk	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	435.1	409.1	400.8	368.0	333.3	299.8
RH 11%	428.9	414.0	405.1	378.4	359.3	316.2
RH 33%	421.7	400.6	402.3	386.6	345.6	307.9
RH 54%	413.6	417.2	410.6	404.0	408.8	378.6
RH 75%	408.3	389.3	391.0	386.3	375.1	372.0



Appendix 7.2 γ-Tocopherol of Total Lipids CL-MFSO and NCL-MFSO

	γ - Tocopherol (µg / g oil) of FSO					
Relative Humidity (%)	1 wk.	3 wks.	5 wks.	9 wks.	13 wks.	17 wks.
RH 0%	476.4	479.7	479.4	451.9	473.4	453.2
RH 11%	475.9	475.0	475.9	444.2	475.3	445.7
RH 33%	463.3	465.4	470.1	447.7	457.2	451.4
RH 54%	452.0	465.1	475.2	449.0	451.4	474.1
RH 75%	466.8	465.7	474.3	450.1	452.7	478.5

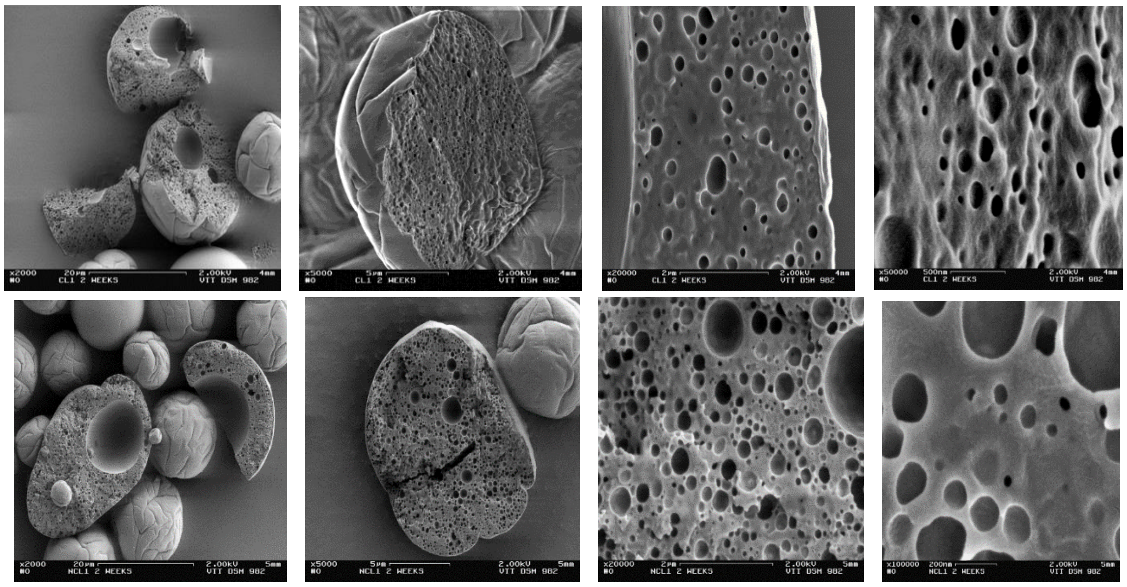


Appendix 7.3 γ-Tocopherol of FSO

Appendix 8. Morphology of CL-MFSO and NCL-MFSO

(a)

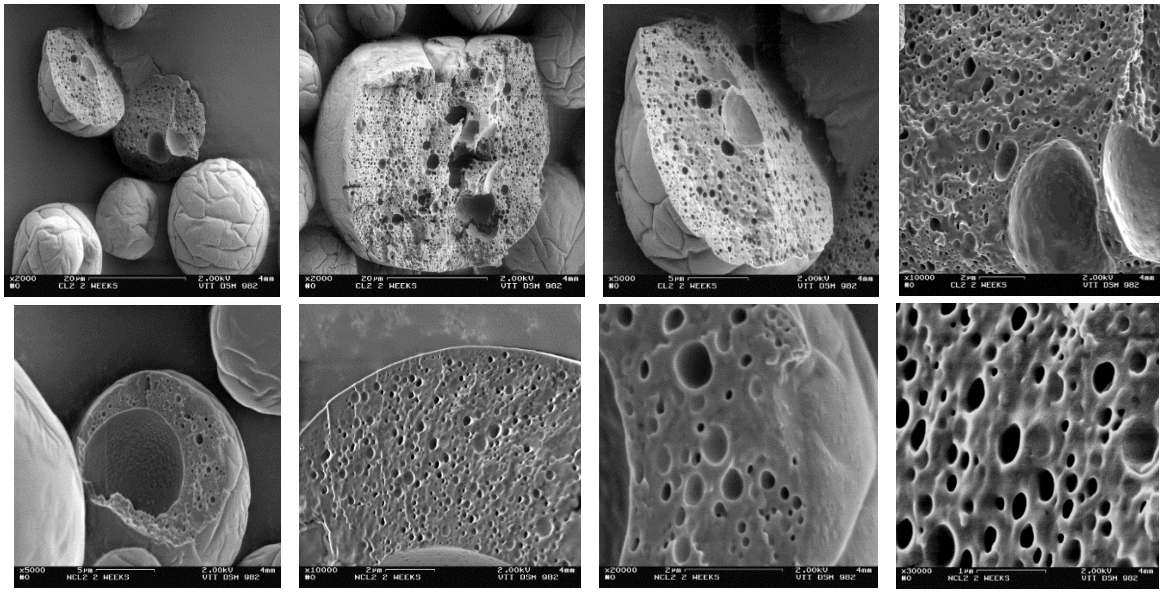
(b)



Appendix 8.1 SEM of cross-linked (a) and non-cross-linked (b) flaxseed oil powder stored 2 weeks @RH 11%

(a)

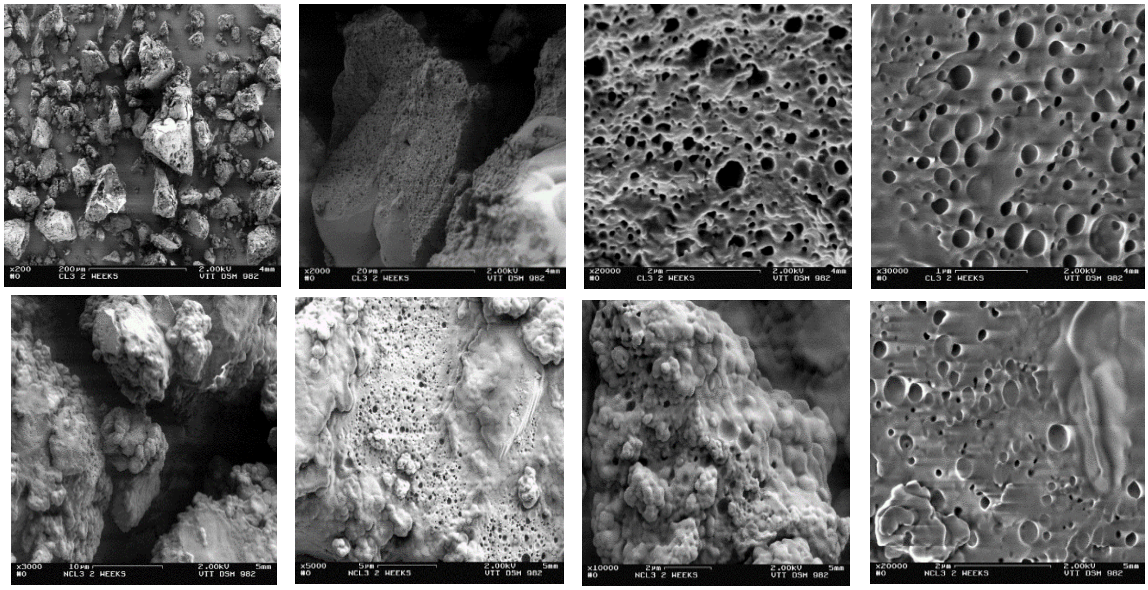
(b)



Appendix 8.2 SEM of cross-linked (a) and non-cross-linked (b) flaxseed oil powder stored 2 weeks @RH 54%

(a)

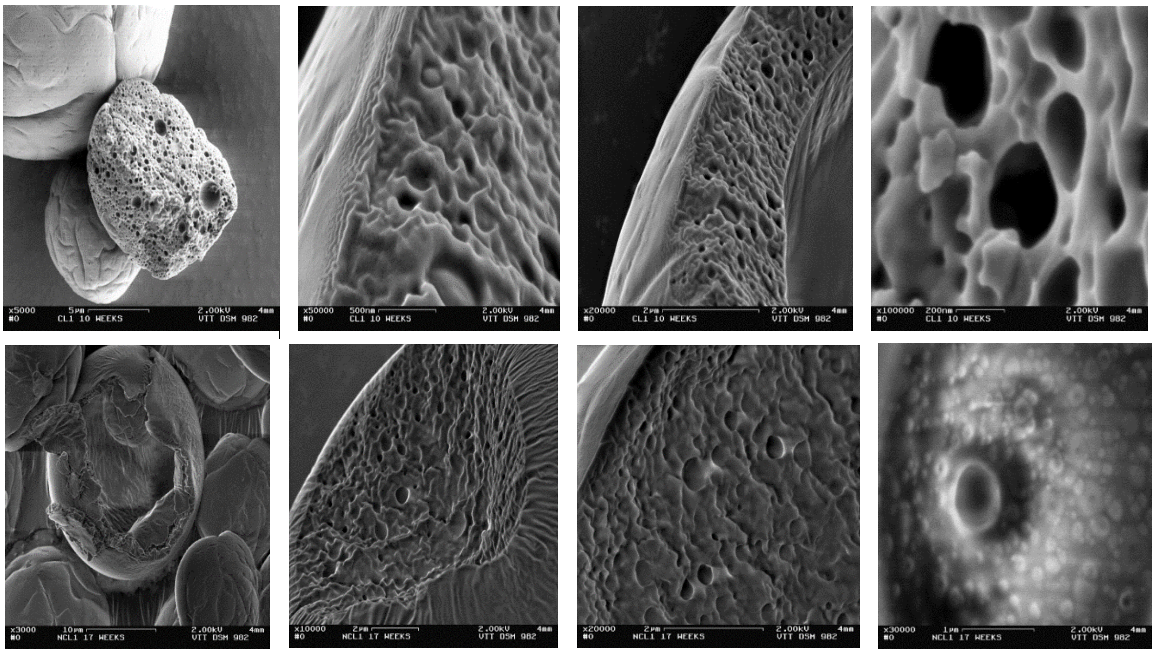
(b)



Appendix 8.3 SEM of cross-linked (a) and non-cross-linked (b) flaxseed oil powder stored 2 weeks @RH 75%

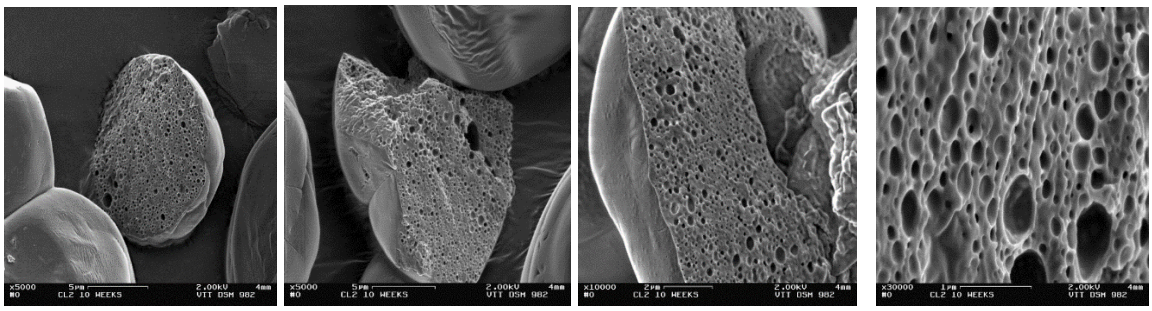
(a)

(b)

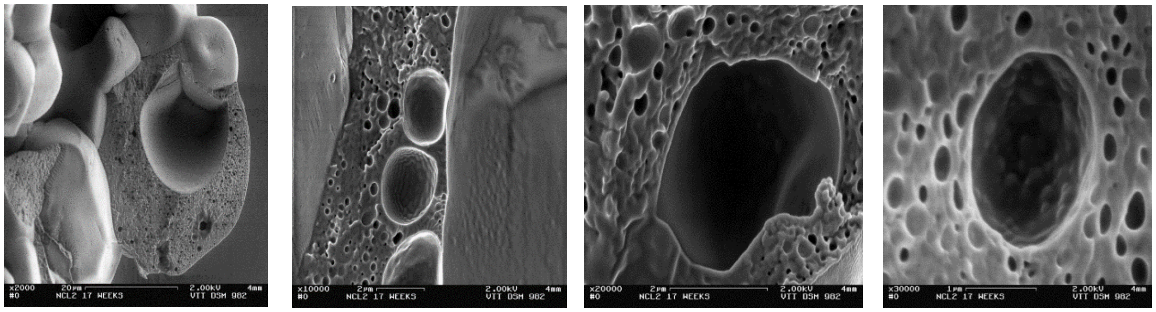


Appendix 8.4 SEM of cross-linked 10 weeks (a) and non-cross-linked 17 weeks (b) flaxseed oil powder stored @RH 11%

(a)

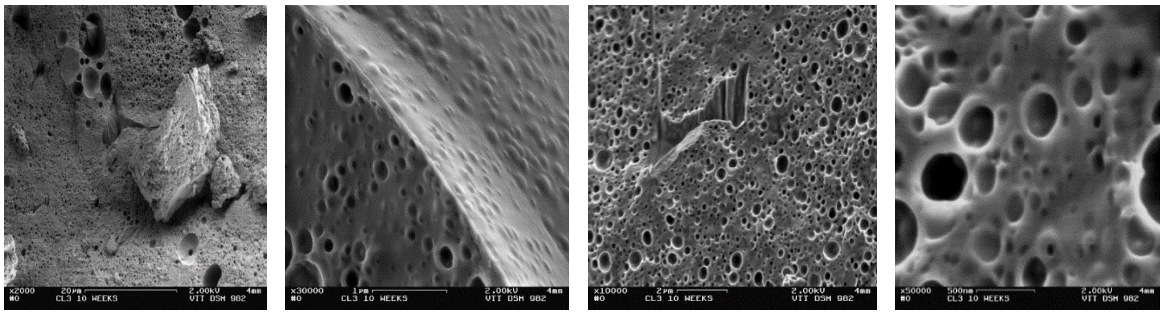


(b)

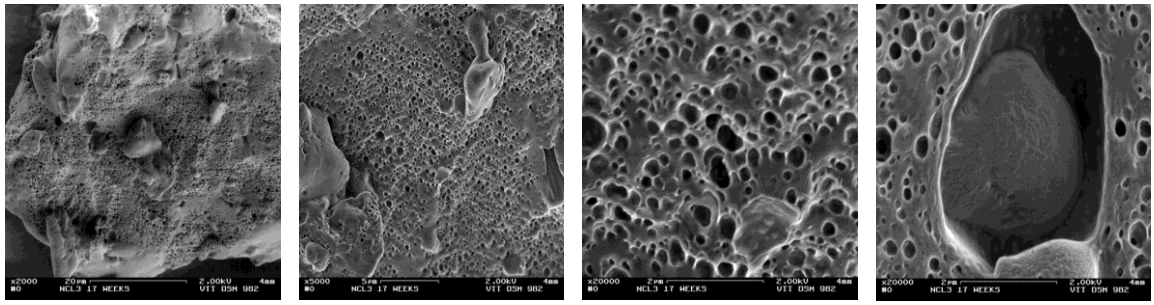


Appendix 8.5 SEM of cross-linked 10 weeks (a) and non-cross-linked 17 weeks (b) flaxseed oil powder stored @RH 54%

(a)



(b)



Appendix 8.6 SEM of cross-linked 10 weeks (a) and non-cross-linked 17 weeks (b) flaxseed oil powder stored @RH 75%